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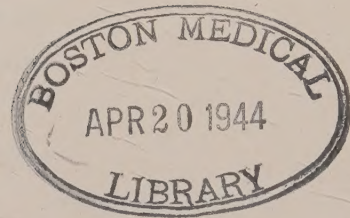
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THE CYTOLOGY OF THE ANTERIOR PITUITARY OF BROODY FOWLS

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TWO PLATES (TEN FIGURES)

The mammalian pituitary has been the subject of considerable study both experimentally and cytologically during all stages of the reproductive cycle. The pituitary of the bird, however, has been neglected, relatively speaking, because our main interest has been in the mammals.

The problem of broodiness with its possible relationships to pregnancy has been scarcely touched. As a result the literature gives us but little information about the cytology of the pituitary of broody fowls. Bell ('19) stated that the cells of the pituitaries of broody hens are least active, that they are mostly small, and that many of them are granular. Riddle, Bates and Lahr ('35) have demonstrated that injections of prolactin induce broodiness. Byerly and Burrows ('36) state that pituitaries of broody hens contain more prolactin than pituitaries of laying hens and males and Burrows and Byerly ('36) found that pituitaries of laying hens and males of broody genetic constitution contain more prolactin than pituitaries of hens and males respectively of non-broody genetic constitution. In their paper of 1938 on broodiness induced by means of environment, Burrows and Byerly conclude that, "Contrary to expectation the pituitaries of broody hens, as compared with those of laying hens, showed no indication of an increase in prolactin-like substance." Here are conflicting statements but it is possible that the pituitary picture is not the same following induced broodiness as it is in the normal course of events.

¹Contribution no. 316.

Because of the difficulty of getting material, this study, to date, has been limited to twenty-one broody hens. Thirteen, two Barred Rocks, one Cornish and ten White Leghorns, were in the early stages of the broody period. One White Leghorn had been incubating for 2 weeks and a second one for 3 weeks. The pituitaries of the other six were removed during the period of motherly care of the young; one Buff Cochin, one White Leghorn and one Barred Rock when young were 1 week old; one White Rock when young were about 4 weeks old and one White Leghorn and one Barred Rock when young were about 7 weeks old.

My studies have demonstrated that in some varieties of fowls (probably all) the pituitaries of broody hens undergo marked transformations and at the end of the broody period or at the onset of another laying period, the return changes are equally marked. In order that the reader may have a better basis for comparison and an understanding of the changes which take place, I wish to describe briefly the pituitary of a laying hen. Perhaps it is best to describe such a pituitary in a general way for variations occur even in pituitaries of laying hens. In general, then, the number of large functional basophiles is high (fig. 6) and they are distributed throughout the gland. The number of both kinds of functional acidophiles is low, comparatively speaking, and their distribution varies much but for the most part one type is found in the posterior half of the pituitary and the other in the anterior half as described by Rahn ('39). These two types of acidophiles were referred to as A_1 and A_2 respectively in my earlier paper (Payne, '42). Many chromophobes are present but it is hazardous to make comparisons. The gland as a whole is larger, particularly in a dorso-ventral direction, than in broody hens. For more detailed and more complete descriptions of the pituitary of fowls see the papers of Rahn ('39) and Payne ('42).

While there are differences between different varieties of fowls, the essential modifications which occur in the pituitaries of hens during the broody period are the same in all studied.

These changes are already present to a greater or lesser extent before the hen begins incubation in Cornish, White Leghorns and Barred Rocks and reach their maximum in White Leghorns during the third week of incubation.

The volume of cytoplasm and of the nuclei in all large basophiles is reduced and the nuclei stain darker than in actively functional cells. The mitochondria show no significant changes but the Golgi substance, following osmication, stands out more conspicuously, is more irregular in shape and smaller in size. All basophiles are probably inactive and their inactivity is correlated with small undeveloped ovaries.

Of the acidophiles in the posterior third of the gland (A_1) some are filled with secretory granules, others are without granules. Most of those with granules appear to be inactive but a few remain active. In the active cells the nuclei are large and clear and the granules are bright scarlet or red. In the inactive cells the nuclei are darker and smaller and the secretory granules of the cytoplasm are a much darker red in contrast with the brighter red granules of the active cells.

Another series of changes, which probably began even before the onset of the broody behavior, is conspicuous. The part of the pituitary first affected lies in the middle dorso-ventrally and about one-third the distance from the anterior end (fig. 5). All cells are involved and the region of change gradually enlarges until practically the whole gland is changed as in White Leghorns. Sometimes the line of change is sharp (fig. 3) or again it may be less so. This line is conspicuous in part because of size differences of the cells, but also because of differences in size and arrangement of the cell cords. In broody hens the cords, even with smaller cells, are larger than in laying hens. Figure 1 is a camera lucida sketch of the cords of a broody hen while figure 2 is from a laying hen. Figure 3 is from a broody hen and shows the line of separation between the large cords with small cells and the smaller cords with large cells. The three figures were drawn to the same scale. In the posterior end of the gland small islands of modified cells may appear but for the most part

changes progress peripherally from the original area. The posterior end of the gland is least affected. Which kind of cell is involved in the initial changes is uncertain, but in the pituitaries studied all changes go on simultaneously.

The acidophiles (A_1), in whatever state of secretory activity they may be found, change as follows: the nuclei become clear and vesicular; the cytoplasm becomes reduced in amount, sometimes to the point where it is scarcely visible in cross sections; and secretory granules are formed.

The A_2 acidophiles, carmine cells of Dawson ('38), which are distributed irregularly in patches throughout the anterior end of the pituitary, change in the same general direction as the A_1 . Before the onset of changes the secretory granules stain dark red, bordering on maroon, and the cytoplasm and nuclei stain darker than in A_1 . The mitochondria are more prominent and sometimes difficult to distinguish from the secretory granules. By means of one or more of these characteristics which may persist for some time, the cells can be followed into late transitional stages. Eventually, however, the identity of the cells is lost as they merge into those derived from the A_1 acidophiles.

The changes in the basophiles proceed toward the same end. The cytoplasm becomes reduced and non-granular and the mitochondria become fewer in number. The nuclei now stain much darker and it is only in later stages that this capacity is changed and a much lighter appearance acquired. I am unable to state positively that these cells secrete acidophilic granules.

The chromophobes may remain unchanged but most of them become modified until they, too, become indistinguishable from the modified acidophiles and basophiles. While it is impossible to follow a single chromophobe through the changes and be sure that the cytoplasm of such cells contains acidophilic granules, so many cells in a given cord contain acidophilic granules it seems reasonable to believe that some of the cells with granules must have come directly from chromophobes. This evidence further indicates that cells which were

originally basophiles and A_2 acidophiles may contain the same acidophilic granules as found in broody cells from other sources.

Figures 7 and 8 are drawings of sections of cords (cell walls shown in part only) of cells at the time when the changes in these particular cords have reached a maximum. In figure 8 (a White Leghorn which had been incubating for 3 weeks) only one cell can be identified by its dark nucleus as basophilic in origin while in figure 7 (a Barred Rock before incubation) there are two such cells. In earlier stages of change many more such modified basophilic cells are present in all cords, and sometimes as in figure 10 all cells are basophiles and chromophobes. A comparison of figure 6 from the pituitary of a laying hen with figures 7 and 8 from the same general region of pituitaries of broody hens, helps to visualize the marked transformation which takes place.

The result of these changes is that a region of the pituitary, almost the whole of it in some cases, becomes modified to the point where all basophiles and acidophiles and nearly all chromophobes have lost their original appearances. Instead, one type of cell dominates almost exclusively. These cells are small in size, due primarily to a reduction of the amount of cytoplasm and in many of them acidophilic granules are present. Seldom, however, is the cytoplasm filled with granules. The nuclei are large, clear, and vesicular, and the cells seem to be actively functioning. The chromatin, present in small flocculent masses, is distributed throughout the nucleus as in the chromophobes and acidophiles. The mitochondria and Golgi materials are similar to those found in chromophobes. For purposes of identification and to avoid repetition of descriptions, I shall refer to these cells as "broody" cells. The maximum degree of change so far observed is found in the White Leghorn hens which had been incubating 2 and 3 weeks. In no other variety studied are the changes as great as in White Leghorns, although Barred Rocks and Cornish are near seconds. Further comparative studies are needed.

The hen cares for her brood for about 7 or 8 weeks, after which she begins to lay eggs again. During the entire broody period, including incubation and care of young, the ovary remains small and the ova undeveloped. The comb is small and colorless. Near the end of the period of motherly care, changes in behavior are noticeable. The young are left to shift more for themselves and the hen may leave them at night for the roost but return to them during the day. The comb becomes more turgid and color returns. Ova begin to enlarge.

The pituitary changes are much as might be expected. In the White Leghorn with chicks between 7 and 8 weeks old, the return was practically complete, and the appearance of the pituitary was similar to that of a laying hen. There were two ova between 10 and 12 mm. in diameter and many smaller ones. The comb was erect and red. In the case of the White Leghorn 1 week after hatching the young, there were little or no indications of change from the pituitaries of hens which had incubated 2 or 3 weeks. Different varieties differ much. The modified region of the pituitary of a Buff Cochin hen 1 week after the young were hatched is very small and the pituitaries from two White Rocks which had incubated eggs for 3 weeks and mothered the young for 3 weeks could not have been identified as pituitaries from broody hens. Of course the pituitaries of these varieties may never have become so highly modified. Additional material is needed for a clarification of this question.

The preceding description of pituitaries of broody hens, inadequate as it is in comparison with direct observations with the microscope, clearly demonstrates that the gland undergoes marked morphological changes during the broody period. There is a recession toward inactivity in both basophiles and acidophiles and the development of broody cells through transformations of other cells. The transformations of the A_1 cells and the chromophobes is certainly direct but with respect to the basophiles and A_2 acidophiles the evidence is not conclusive. Probably these cells first change into chromophobes and then into broody cells. In working with transitional stages

one cannot draw lines sharply between the chromophobes and secretory cells and vice versa.

What is the significance of these changes? Do they merely accompany the broody behavior or could they be either directly or indirectly the cause of it? The experimental evidence seems conclusive that injections of prolactin are capable of inducing broodiness in fowls and at present I am inclined to suggest that the broody cells, since they are actively secreting, may be concerned with prolactin production. I make this suggestion even though broodiness in fowls can be induced and concluded by changing the environment and even though the experimental evidence is conflicting as to the prolactin content of pituitaries from broody hens in comparison with those from non-broody hens. Prolactin content, however, is not necessarily a measure of prolactin release. If the broody cells do secrete prolactin and if prolactin causes broodiness in the normal cyclic changes of the fowl, there still remains the unanswered question as to the cause of the pituitary changes.

There are dangers even in suggestions. Broody cells cannot be the only source of prolactin because extractions and assays demonstrate its presence at other times. Possibly we have in the broody cells nothing more than a method of acceleration of production.

Among the varieties of fowls studied there is considerable variation as to the degree and extent of pituitary modification and the greatest change was found in the White Leghorn. Why these differences if the broody cells are concerned in the secretion of prolactin and why is the White Leghorn pituitary modified more than others? At first it seemed that the answer might be found in the differences among varieties with respect to broody behavior. White Leghorn hens become broody very seldom. They have been bred for egg production and broody hens are discarded from the flocks. Other varieties, such as Cornish, become broody more frequently. As Cornish fowls, in the pre-incubation period at least, have pituitaries modified about as much as do White Leghorns, there is no correlation between the frequency of the broody

condition and the degree of modification. Possibly the answer may be found in the degree of sensitivity of the different varieties of fowls rather than the quantity of hormone produced.

In all varieties of fowls egg laying is discontinuous. What is the cytological picture of the pituitary during these non-laying intervals? Recognizing that there might be similarities between the pituitaries of broody hens and those of hens in the quiescent period, I examined the pituitaries of two hens which had recently moulted. The ovaries were small, similar to those of broody hens. In one the ova were about 1 mm. in diameter; in the other the largest ova were about 4 mm. The acidophiles of the posterior end (A_1) were more numerous than in broody hens and were larger and more granular. The nuclei were large and stained lightly as in actively secreting cells. The A_2 were similar to those of laying hens. As to be expected, the basophiles, while abundant, had receded in size and had the appearance of inactive cells. There were many small chromophobes but no indications of broody cells. It seems probable that broody cells are found only in broody hens.

Perhaps no attempt should be made to compare reproductive activities and behavior in mammals and fowls. Nevertheless questions do arise even though we attempt to push them aside. If phyletic relationships between birds and mammals exist, even though remote, then some of the fundamental processes should be similar if not identical. Certainly the development of the sex glands, the growth and development of the germ cells, and ovulation are essentially the same in the two groups. The endocrine glands in the two groups are very similar in structure and activity and endocrine physiological processes may be expected to be closely comparable. Variations such as the number of ovulations per year, the interval between ovulations and the estrous cycle are probably not significant because of the wide range found within both birds and mammals. Fundamental differences are the retention of the ovum or ova within the uterus and the development of placentae and

corpora lutea in mammals. Perhaps even this difference is not so significant when we take into consideration the fact that a few mammals lay eggs, that placentation may be nothing more than a close contact between the chorion and the uterine wall, and that some fishes and reptiles retain the ova during embryonic development and have a yolk-sac type of placenta. Luteal tissue has also been described in the bird and shark, although the actual homology is still debatable.

If there is any period in the fowl comparable to gestation in mammals, it must be the period of incubation. During gestation the mammary glands develop, resulting in milk secretion. Although in the fowl there is nothing comparable, we do find a similar secretion in the crop-glands of pigeons and in both mammal and fowl motherly behaviors are closely alike.

I shall make no attempt to develop the idea of comparisons and homologies further than to point out that if special cells develop in the pituitaries of mammals during pregnancy and if these cells are acidophile-like cells as Severinghaus ('37) believes, then the broody cells of fowls may be homologous to the "pregnancy" cells of mammals.

The changes which have been described in the pituitaries of broody fowls again emphasize the remarkable degree of plasticity which the pituitary possesses. Secretory cells may become active or inactive and change morphologically, depending upon the stimuli received. Changes can be produced experimentally by castration, by limited diet, by injections of both estrogens and androgens, by light, by thyroidectomy, and by other means. Perhaps such plasticity is in some degree correlated with the many functions ascribed to the pituitary.

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PLATE 1

EXPLANATION OF FIGURES

- 1 A small section from the mid-region of pituitary of a broody hen showing size and arrangement of cords.
- 2 A drawing from the same region as figure 1 of a pituitary of a laying hen. Note the smaller size of the cords even though the cells are larger.
- 3 A drawing of the cords from the pituitary of a broody hen showing sharp line of separation between modified and unmodified regions.
- 4 An outline sketch of a saggital section of the pituitary of a laying hen.
- 5 An outline sketch of a saggital section of the pituitary of a broody White Leghorn hen which had been incubating 3 weeks. A is the anterior, and P the posterior end and N the dorsal or neural side. With the exception of a small region at the posterior end limited by a broken line, the entire gland was modified. The small area near the anterior end enclosed with a broken line is the region where changes first start.

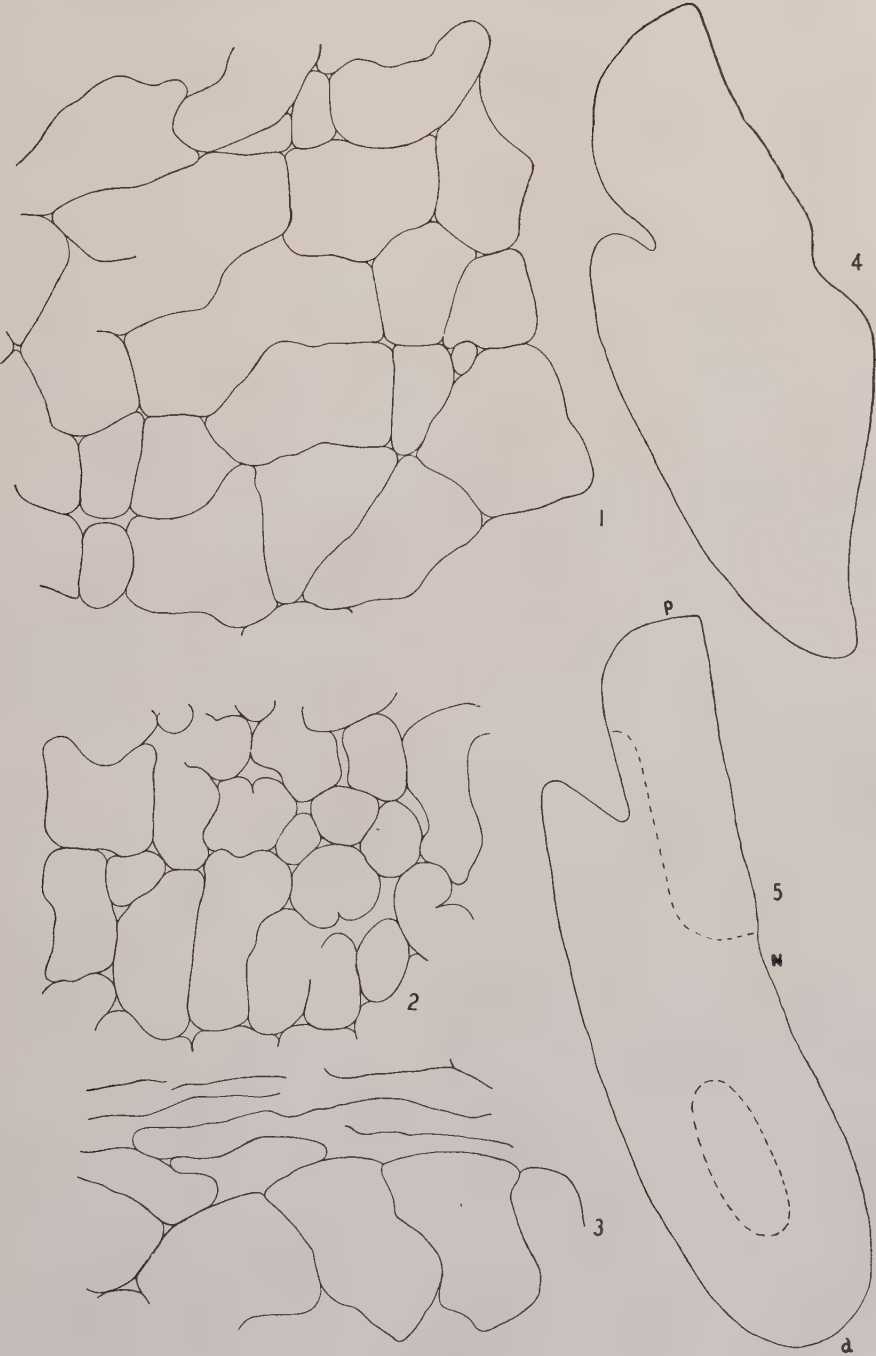


PLATE 2

EXPLANATION OF FIGURES

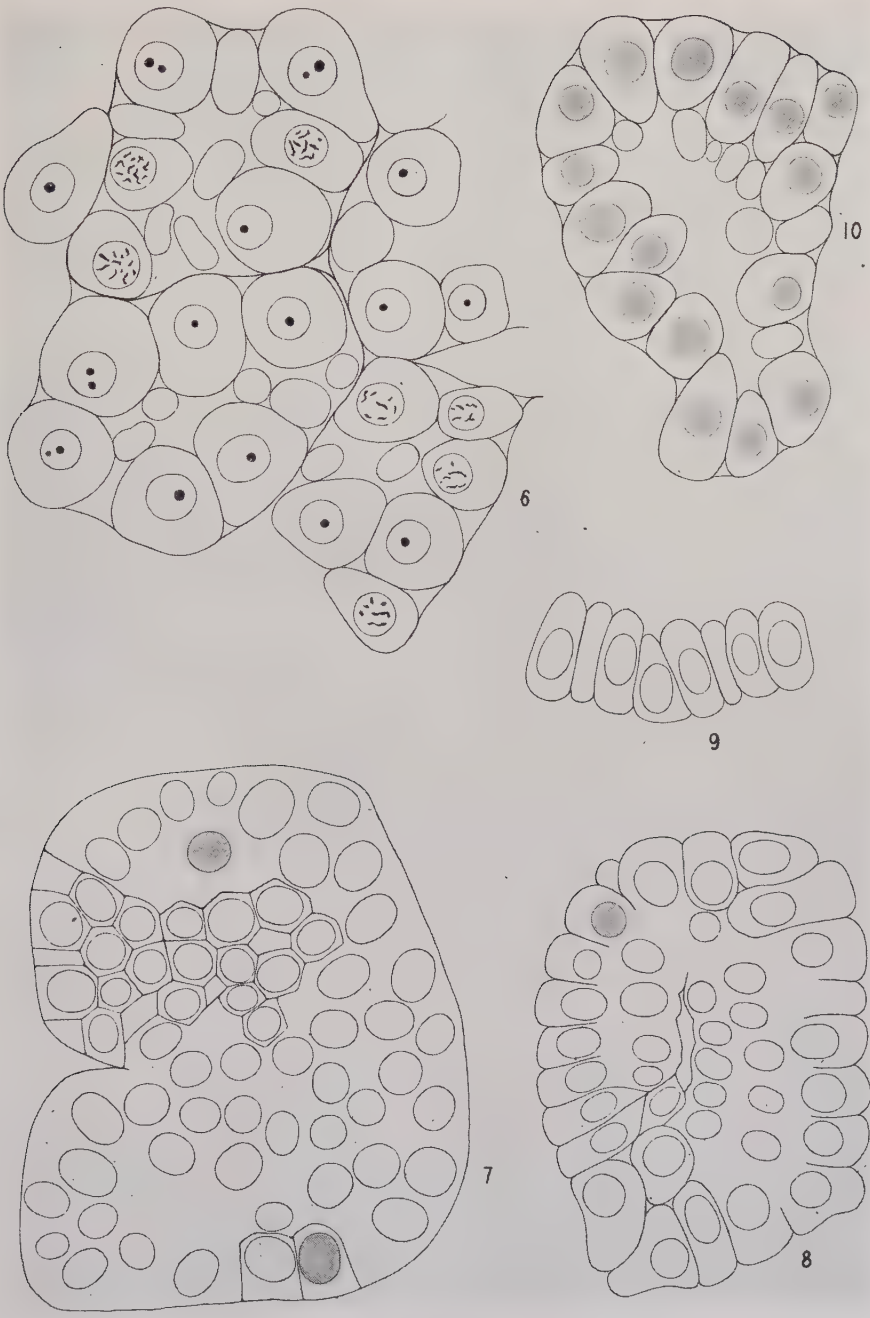
6 A drawing from the pituitary of a laying hen showing numerous large basophiles (cells with spherical nucleoli) a lesser number of acidophiles and the chromophobes. Section taken from a region slightly nearer the posterior end and in the middle dorso-ventrally.

7 A section of the broody cell region of a pituitary from a Barred Rock broody hen. Only a few cell walls are drawn. All cells with the exception of two with dark nuclei look alike. The two with dark nuclei are basophilic in origin.

8 A section of the broody cell region from the pituitary of a White Leghorn hen during period of incubation. The cells are similar to those in figure 7 but slightly smaller. Only one dark nucleus is present here. Not all cell walls are drawn. Contrast this figure with figure 6 from the same region of a laying hen. All figures on this plate drawn to the same scale.

9 A few broody cells cut longitudinally.

10 A section of a cord of cells from the pituitary of a Barred Rock hen in the early stages of the broody period before cellular transformations were completed. With the exception of the small chromophobes, all cells have dark nuclei and are changing basophiles. Sections of cords without some acidophiles are rare.



THE BLOOD SUPPLY AND INNERVATION OF THE CHOLEDOCHODUODENAL JUNCTION IN THE CAT

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FOUR TEXT FIGURES AND THREE PLATES (EIGHT FIGURES)

In preparation for experimental studies on the nervous regulation of bile flow in the cat¹ — one of the few animals in which the rate of emptying of the biliary tract has been studied quantitatively — it became necessary to determine the origin, course and relations of the finer nerves and arteries supplying the sphincter of Oddi and contiguous areas of the duodenum.

METHODS

The vascular pattern was revealed by injecting the coeliac artery with Ward's liquid latex (a rubber solution colored with a bright red dye) diluted with an equal volume of distilled water. The technique has been described by Gamble ('39). After complete injection of the artery ligatures were tied around the lower esophagus, the middle of the duodenum and the root structures at the porta hepatis. A fixing solution of 5% formalin and 1½% glacial acetic acid was then injected into the tied-off portion of the gastrointestinal tract until the stomach was moderately distended. Meanwhile additional fixative was poured over the external surface of the tract. After 15 minutes the rubber was sufficiently set to permit trimming of the specimen. Then it was removed, pinned out over night under fixative and subsequently carried through alcohol and benzol to a solution of 5 parts of methyl salicylate and 3 parts of benzyl benzoate. Thus rendered translucent, the finer radicals could be studied under a dissecting microscope.

¹ See Johnson and Poyden ('43).

It was soon noted, however, that the red dye in the latex was dissolved by the alcohols. Accordingly, India ink was substituted, the injection mass being made up in the following proportions: latex, 2 parts; distilled water, 3 parts; India ink, 1 part.

The nerve supply was demonstrated by two methods: (1) gross dissection of fresh specimens — a procedure which is limited to a few hours after death, since thereafter the finer nerves undergo cytolysis; and (2) teasing methods — dissection with needles under a binocular microscope after specimens had been fixed and stained by the Wharton-Sihler technique. The latter method is as follows:

Spread fresh tissues on paper and partially dry.

Fix in solution "A" for 18 hours

glycerine — 1 part

glacial acetic acid — 1 part

1% aqueous chloral hydrate — 6 parts

Stain in solution "B" for 24 hours

glycerine — 1 part

Ehrlich's hematoxylin — 1 part

1% aqueous chloral hydrate — 6 parts

Store specimen in pure glycerine

Decolorize (if necessary) in 70% alcohol + 1% HCl

or in solution "A."

Sihler's method, discovered in the files of the late Franklin P. Mall and reviewed by Wharton for the purpose of studying the innervation of the human ureters ('32) and the female reproductive organs of the monkey ('37 b), is especially adapted for studying the nerve pathways that lie in thin sheets of tissue such as the lesser omentum. The latter can be stretched out, fixed and stained in a fluid medium that permits dissection of a given region at leisure.

While Wharton recommended that the fresh membranes be spread out on paper ('37 a) it was the experience of Doctor Schulze, who made the dissections shown in plates 2 and 3, that the specimens came off the paper and contracted considerably after being placed in solution A. Therefore the specimens were pinned out on a flat disc of paraffin.

A second refinement of the technique was made in the destaining methods. It was found that decolorizing the specimen long enough to render visible finer nerve plexuses in the duodenal wall bleached the more exposed nerves in the omentum. Accordingly the process was localized by dropping acid alcohol through a pipette on just the area which was overstained and by tilting the dish so that the solution did not reach the rest of the specimen. Furthermore this method proved to be highly selective, since the nerves retained the dye longer than the hollow vessels of the duodenal wall. Similarly, if decolorization was excessive, restaining could be controlled by tilting the dish so that the staining solution covered only the duodenal segment. To further increase the translucency of the duodenal wall the mucosa and most of the submucosa was scraped off. With these modifications of the technique it was possible to demonstrate the finer ramifications of the nerve plexus in the choledochoduodenal region.

OBSERVATIONS

The blood supply of the choledochoduodenal junction

Figure 5 is a photograph of a cleared specimen of stomach and duodenum that has been injected with liquid latex. The duodenum has been rotated counter-clockwise (as viewed from the stomach), and the common bile duct and portal vein have been pulled up and to the reader's left, in order to expose the dorsal side of the duodenum and the under side of the duct. The arteries appear white, the portal system (and the duct) black.

The distribution of vessels resembles, in general, that of the human, but the pattern reveals the greater mobility of the viscera — the coeliac artery (cf. fig. 2) being nearly $1\frac{1}{2}$ inch long. In figure 5 it is shown tied at the point where it branches into its three main divisions, the left gastric, the splenic and the hepatic artery. The coronary vein, on the lesser curvature, assumes the course found in some human anomalies, i.e., it drains down the lesser curvature, to enter the portal

vein, instead of up the lesser curvature toward the esophageal end.

Of special interest, for the purpose in hand, is the gastroduodenal artery and its first large branch, the superior pancreaticoduodenal artery. Just as the latter leaves the parent vessel (fig. 5) it finds its course impeded by the common bile duct. Accordingly, its first few duodenal rami branch from a common stem—here designated the dorsal common duodenal artery (*A. duodenalis communis dorsalis*)—which passes to the left of the bile duct, whereas the more distal duodenal rami come off that part of the superior pancreaticoduodenal artery which passes to the right of the bile duct.

In the specimen shown in figure 5, three proximal duodenal rami (1, 2, 3) arise from the common duodenal artery. However, it will be noted that these rami are also connected with the main pancreaticoduodenal vessel by anastomoses passing across the bile duct just before it enters the intestinal wall; so that occasionally the third branch of the common duodenal artery is taken over by the main artery. Also, the first branch may arise independently.

This is the case in the specimen shown under higher power, in figure 6. This detailed figure—drawn instead of being photographed, in order to preserve the effect of depth—reveals the fact that the intramural part of the bile duct (see segment to left of asterisk) is supplied by branches of the third, fourth and fifth dorsal duodenal rami. These sink down from the serosa (where they appear blackest) through successive layers of the intestinal wall to the surface of the ampulla of Vater, there to unite in capillary nets.

This circulatory pattern is quite different from that described by Dardinski ('35), who reported that the arteries accompanied the duct through the choledochal window of the intestine. To be sure there are a few such branches in the cat (to right of asterisk, fig. 6) but in the main the papilla seems to be more nearly an integral part of the duodenum; wherefore to be supplied like any other portion of the gut by arteries that dip down from the serosal surface. This may be

due to the fact that in the cat (as in the dog) the musculature of the duodenal wall is much more intimately related to the intramural portion of the duct than in man (Boyden, '37, and unpublished data). Whatever the interpretation, it appears that in the cat the principal arteries to the major papilla do not enter the intestine with the bile duct and its nerves.

The gross innervation of the choledochoduodenal junction

For purposes of general orientation this study was initiated by gross dissections of fresh specimens. While this method had obvious disadvantages — namely the nerves had to be identified before cytolysis occurred, it was not always possible to distinguish fine nerves from delicate vessels, and it was evident that what appeared to be single trunks must have been closely woven plexuses — nevertheless it revealed the existence of a hitherto unrecognized nerve, directed toward the pylorus, which is herewith designated the gastroduodenal nerve.

As shown in figure 1, it lies to the left of the artery of that name and divides into two main branches. One of these follows the right gastroepiploic artery and the other — here designated the choledochoduodenal nerve — ends in the acute-angled space bounded by the choledochoduodenal junction. If this be traced back to the parent stem (the gastroduodenal nerve) the latter is seen to arise as a confluence of two trunks, one a branch of the hepatic plexus and the other a branch of the coeliac division of the dorsal (s. right) vagus nerve.

A second specimen (fig. 2) confirms this origin and displays the gastroduodenal nerve from the right side. In the upper part of the figure a segment of the portal vein has been removed to expose the gastroduodenal nerve as it passes first to the left of (i.e., deep to) the hepatic artery and then to the left of the gastroduodenal artery. Following the nerve in the reverse direction (i.e., toward the diaphragm) it can be seen to join two hepatic branches of the right coeliac ganglion, one of which (A) receives the coeliac branch of the dorsal (right) vagus nerve, the other of which (B) receives (at X) contributions from the left coeliac ganglion.

It would thus appear from gross dissection of fresh specimens that the choleldochoduodenal ramus of the gastroduodenal nerve might contain fibers from the dorsal vagus and from both coeliac ganglia, although fibers from the right side would seem to predominate.

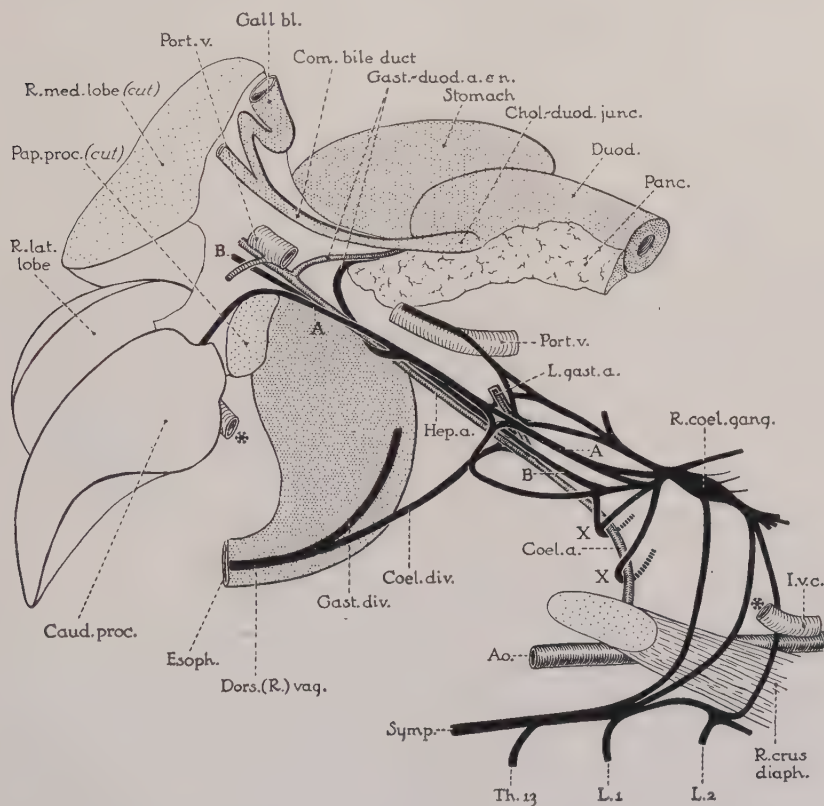


Fig. 2 Dissection of coeliac trunk and associated nerves in fresh specimen as viewed from the right side to show origin of gastroduodenal nerve (E. A. Boyden). The inferior vena cava has been severed (the separated cut ends are indicated by two asterisks), the diaphragm has been cut and the liver moved to reader's left thereby exposing right side of stomach and coeliac division of dorsal (s. right) vagus nerve. XX, communications between right and left coeliac ganglia around anterior surface of coeliac artery. A, bundle of fibers running from right coeliac ganglion to hepatic plexus where it is joined by bundle from coeliac division of dorsal vagus. Half way to the liver the plexus gives off the gastroduodenal nerve. Th.13, L.1, L.2, rami communicantes of right thoracolumbar chain, the latter giving off three splanchnic nerves.

A second avenue of approach to the choledochoduodenal junction, as revealed by dissection of fresh specimens, is a diffuse plexus of nerves surrounding the gastroduodenal artery, herewith designated the gastroduodenal plexus. As shown in figure 1, it gives off fibers which pass directly to the upper common bile duct and other fibers, lower down, which course along the duodenal branches of the superior pancreaticoduodenal artery to enter the same acute-angled space between the bile duct and the duodenum to which the choledochoduodenal branch of the gastroduodenal nerve has been traced.

The fibers of this plexus are derived from the hepatic plexus and possibly from the coronary nerve of the lesser curvature — a bundle swinging across the gastrohepatic ligament from left to right. This nerve appears to be made up of fibers from the left gastric plexus and ventral vagus which ascend the right gastric artery. In the specimen shown in figure 1, the coronary nerve appears to cross the gastroduodenal nerve superficially, but in many specimens the greater portion of its fibers pass deep to it.

Analysis of stained specimens

General observations. Figure 3 is a photograph of the omentum and gastropancreatic folds of an immature animal after the peritoneum, lymph nodes and veins have been removed. Immature animals were found to be unsuitable for detailed dissection because of their fragility. However, this particular figure is included to show the relationship of nerves to arteries. It will be seen that in general the sympathetic plexuses follow the vessels very closely, whereas vagal branches are more apt to wander separately through the omentum. While this dissection was incomplete, every possible effort was made to preserve all nerves in the specimens photographed in figures 7 and 9, and one can say with certainty that these figures give a comprehensive picture of the general distribution of nerves to the common bile duct, duodenum

and stomach, and that at least none of the larger nerves to these organs have been destroyed.

The coronary nerve of the lesser curvature. In looking at dissections of the lesser omentum and gastropancreatic folds one is first struck by the fact that the large central area is devoid of blood vessels and nerves (figs. 3 and 7) and then by the fact that this area is circumscribed by a ring of nerves. The lower half of this ring is formed by the "nervus coronarius curvaturae minoris" of Valentin (1841). This small but constant nerve issues from a union of ventral vagus fibers with the sympathetic fibers of the left gastric plexus at about the angular incisure of the stomach. Passing from left to right in close association with the gastric arterial arch (C.n., fig. 3), it then ascends with the right gastric artery until it is lost by anastomoses in the plexuses surrounding this vessel and other adjacent arteries. Some of its fibers can be traced to the upper portion of the bile duct and into the hepatic plexus (figs. 4, 8 and 10). In most specimens (figs. 8, 9 and 10) it runs deep to the gastroduodenal nerve (Gd.n.), but in others it lies superficial to it (figs. 1 and 4).

Of special interest, from the standpoint of the composition of this coronary nerve, is the specimen shown in figure 9. What might be interpreted as a segregated vagal portion of the nerve crosses the omentum some distance above the lesser curvature as an independent nerve (An.). On attaining the left side of the right gastric artery it divides into a descending branch, which communicates with the coronary nerve, and an ascending branch, crossing superficial to the gastroduodenal nerve, which anastomoses with the beginning of the gastroduodenal plexus. Several of these fibers can be traced to the common bile duct. This suggests that the coronary nerve may normally contribute ventral vagus fibers to the gastroduodenal plexus.

This finding is contrary to Kollman's description of the cat (1860). He maintains that the fibers of the ventral vagus in this nerve do not ascend with the sympathetic nerves along the right gastric artery but separate from them and descend

to supply the pyloric end of the stomach and the first part of the duodenum.

Stowell (1881) and Alexander ('40) make no mention of the coronary nerve. McCrea ('24) describes it in the cat but does not name it. He states that it sends a series of branches

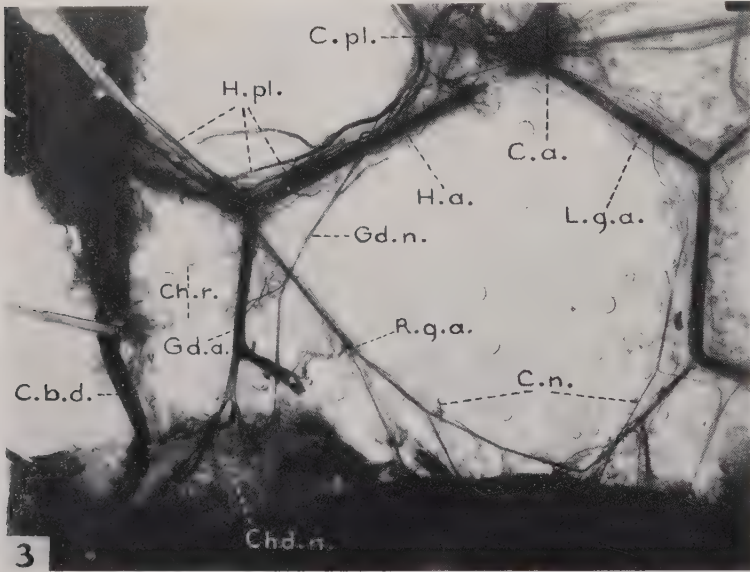


Fig. 3 Photograph of dissection of stained preparation of lesser omentum and gastropancreatic folds in a half-grown cat ($\times 1\frac{1}{2}$). Arteries retained to show relationship to nerves and their differing appearance. C.a., coeliac artery surrounded by coeliac plexus (C.pl.); L.g.a., left gastric artery and plexus; H.a., hepatic artery and plexus (H.pl.); R.g.a., right gastric artery and coronary nerve (C.n.); Gd.a., gastroduodenal artery in region where it has been stripped of its plexus and where it is forking into superior pancreaticoduodenal and R. gastroepiploic arteries; Gd.n., gastroduodenal nerve; Ch.r., two choledochal rami of gastroduodenal plexus, stretched by retraction of bile duct (C.b.d.); Chd.n., choledochoduodenal ramus of gastroduodenal nerve (cf. fig. 1).

downward to the pyloric antrum and a few fine "hepatic branches" upward toward the liver, one of which he calls a "pyloric branch." His diagrams, however, picture the hepatic rami as coming off near the gastric incisure, then ascending in the gastrohepatic omentum to the porta. He does

not mention or show the continuation of the nerve into the plexus of the right gastric artery.²

The significance of this nerve from the standpoint of the present paper lies in the fact that through it a few ventral

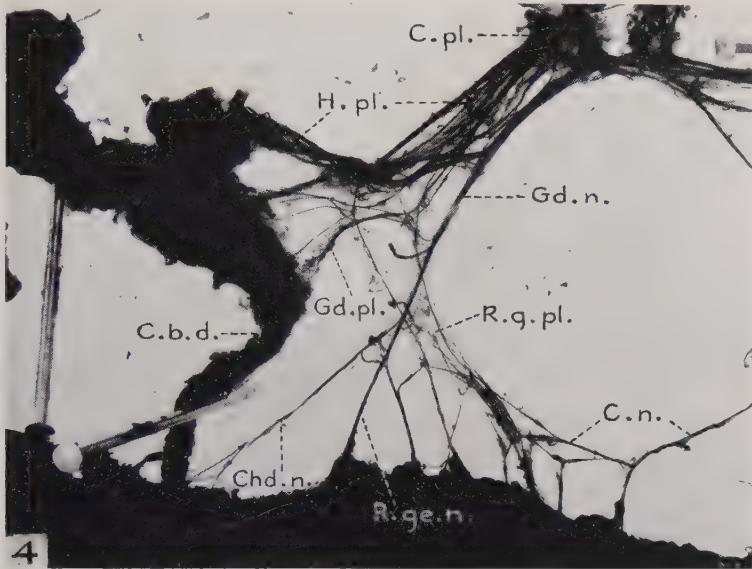


Fig. 4 Photograph of stained preparation ($\times 2$). Arteries removed together with finer plexuses to display gastrooduodenal nerve (Gd.n.), its relation to hepatic plexus (H.pl.) and its subdivision into choledochoduodenal (Chd.n.) and right gastroepiploic (R.ge.n.) nerves. Note strands of gastrooduodenal plexus (Gd.pl.) leading to common bile duct (C.b.d.). C.pl., coeliac plexus; R.g.pl., right gastric plexus.

² McCrea's principal studies were made on the human newborn and on the dog. In the latter species, according to McCrea's drawing, the sole hepatic branch of the ventral vagus leaves the main nerve at a point corresponding to that of a small nerve, in figure 1, labelled Hep.br. This traverses the lesser omentum superficial to the papillary process of the caudate lobe until it reaches the porta hepatis. There it divides into ascending branches, which supply liver and gall bladder, and descending branches which follow the bile duct to the pyloric region. By stimulating such superficially placed hepatic branches in the dog, Chiu ('42) was able to cause contraction of the gall bladder.

In the cat, on the contrary, the hepatic branches of the ventral vagus reach the hepatic plexus either by traversing the coronary nerve or by entering the left gastric plexus which passes deep to the papillary process (figs. 7 and 9). By severing the ventral vagus in the cat Johnson and Boyden ('43) were able to retard the emptying of the gall bladder, but more marked effects were obtained by severing the dorsal vagus.

vagus fibers attain the gastroduodenal plexus from which they may pass as choledochal rami to the upper part of the common bile duct (or to the gall bladder). The number of such fibers is, however, very small.

The gastroduodenal plexus. As the gastroduodenal artery leaves the hepatic artery (fig. 1) it is invested with a sheath of fine and intermediate-sized strands from the coarse nerves of the hepatic plexus. Distal to this point on the hepatic artery other fibers from the hepatic plexus pass into the space between the gastroduodenal artery and the bile duct. These two somewhat arbitrarily defined plexuses (cf. Gd.pl.¹ and Gd.pl.², fig. 8), together with a few recurrent fibers of the coronary nerve, constitute the gastroduodenal plexus—a term designed both to show the relation of the plexus to the artery and to distinguish it from the single trunk (the gastroduodenal nerve) which lies well to the left of the artery (Gd.n., fig. 3). At the termination of the artery, part of the plexus follows the superior pancreaticoduodenal artery and part the right gastroepiploic artery. But all the way down, fine choledochal rami are given off to the bile duct. These are especially well shown in figure 10 (Ch.r.). (See also figs. 8, 4 and 3.)³

The gastroduodenal nerve. This is a definitive nerve forming the right upper arc of the nervous ring which surrounds the central area of the omentum. Its composition, as displayed in stained specimens, is best shown in figures 9 and 10 (Gd.n.). These clearly indicate that the nerve receives fibers both from the dorsal vagus (primarily from its coeliac division, C.d. and 1, fig. 9, but also from its gastric division, G.d. and 2,

³ Judging from three experiments in which no effect on the rate of emptying of the biliary tract was observed when the gastroduodenal plexus escaped section but the gastroduodenal nerve was cut (Johnson and Boyden, '43), it is probable that these choledochal rami of the plexus do not convey efferent fibers to the musculature of the choledochoduodenal junction but rather are composed of afferent and vaso-motor neurons. It is possible, however, that fibers of the choledochoduodenal branch of the gastroduodenal nerve may occasionally traverse the adjacent plexus. Such a course is suspected in the specimen shown in figure 8. Therefore in seeking to eliminate the gastroduodenal nerve, experimentally, it has been the practice to cut all roots of the gastroduodenal plexus.

fig. 9) and from the right and left coeliac ganglion (fig. 10). Here, in fact, three trunks (two large and one small) can be seen passing up into the coeliac plexus from the main nerve (Gd.n.). It would thus appear that the bulk of its fibers are of sympathetic origin.⁴

After the gastroduodenal nerve leaves the hepatic plexus it divides into choledochoduodenal and gastroepiploic trunks. This point of division is variable. It may be placed high up in the right gastropancreatic fold (as in fig. 10), or extremely low down (as in figs. 1 and 3) or, more commonly, on a level with the upper third of the bile duct (figs. 4, 8 and 9). Its choledochoduodenal branch—the one which merits special attention—may pass directly to the bile duct and descend to the junction along the left side of the duct (figs. 4, 9 and 10) or enter the choledochoduodenal angle from the pyloric side (figs. 1 and 3).

As the gastroduodenal nerve descends to the left of the common bile duct, it gives off a series of choledochal rami to the middle segment of the duct. These may branch from the main nerve about the time it divides (fig. 9) or from its continuation, the choledochoduodenal nerve (Chd.n., fig. 10). All these, together with the choledochal rami derived from the gastroduodenal plexus, anastomose freely to form a para-choledochal plexus on the left outer surface of the bile duct (figs. 8, 11 and 12).

The intramural-choledochal and myenteric plexuses at the choledochoduodenal junction. The choledochal rami and duodenal rami of the choledochoduodenal nerve and superior pancreaticoduodenal plexus penetrate the walls of the common

⁴ Experimentally, it can be shown that section of this nerve produces marked delay in emptying the biliary tract by eliminating the dorsal vagal components that pass through it to the choledochoduodenal junction (Johnson and Boyden, '43). Apparently its sympathetic components are not involved in regulating the flow of bile at the duodenal orifice, for section of all splanchnics only slightly accelerates the flow of bile, if at all, and this slight acceleration may be attributed to removal of the sympathetic inhibitory pathway to the gall bladder. Therefore, the sympathetic components of the gastroduodenal nerve are presumably afferent or vaso-motor fibers or are destined for the pancreatic, gastric or duodenal rami of the nerve.

bile duct and duodenum, respectively, and there connect with intrinsic nerve nets (figs. 11 and 12). The finer-meshed plexus of the bile duct consists of slender, round fiber-bundles with very small ganglia appearing as barely perceptible thickenings at some of the nodal points (Intramur.pl.).

The myenteric plexus (of Auerbach) lies between the longitudinal and circular muscle tunics of the duodenum and consists of broad, flat fiber-bundles with large, angular ganglia at the nodal points.

The relationships of these plexuses and of the extrinsic nerves at the choledochoduodenal junction have never been adequately described. These preparations (figs. 11 and 12) show that fiber-bundles of small size (Conn.) connect the intramural-choledochal with the myenteric plexus at the level of the window through which the bile duct enters the duodenum. A few transitional ganglia (Tr.gangl.), intermediate in size between those of the myenteric plexus and the intramural choledochal plexus, are located at the margin of the window. The fiber-bundles emanating from these ganglia communicate with both plexuses. The difference in size of the two nets and the fact that the finer net accompanies the duct through the window, indicate that the choledochal plexus is not to be considered as merely an extension of the myenteric plexus onto the duct, as some authors would lead one to suppose.

Our studies have not carried the choledochal plexus beyond the point where it enters the window in the duodenal wall. Few investigators have done so. Variot (1882), working at a time when little was known about the musculature of the biliary tract, examined sections of the choledochoduodenal junction of the dog and described a plexus within the muscular layer of the common bile duct which contained ganglia that increased in size and number as the duodenum was approached. He stated that in the duodenal wall where the musculatures of the duodenum and common bile duct met, Auerbach's plexus was directly continuous with the intermuscular plexus of the common bile duct. It must be remembered, however, that he considered the muscularis of the bile duct to consist

of an inner circular and outer longitudinal layer, which was much thinner but nevertheless directly continuous with the corresponding layers of the duodenum—a concept which cannot now be accepted. Nor did he recognize the presence of a special sphincter muscle in this region. Variot also described a collar of fairly large ganglia situated around the base of the ampulla at the level of the submucosa of the duodenum. He considered these ganglia as belonging to the submucous plexus of Meissner and postulated that they were connected with fibers to the muscularis mucosae and the surface epithelium. Although he examined only the dog, Variot maintained that similar relations should hold in man.

Oddi and Rosciano (1895) also described this collar of ganglion cells in the dog. In addition, they stated that fibers, apparently arising from these cells, formed an abundant network in the sphincter muscle. They maintained, however, that these ganglia are not related to the ganglia of Auerbach's or Meissner's plexus, but are specialized structures, which, under the control of a spinal center, are involved in maintaining the tonicity of the sphincter muscle.

Dardinski ('35) in a study concerned primarily with the configuration and musculature of the intramural part of the common bile duct in man, made a more or less incidental observation concerning the nerve supply of the papilla. He saw white glistening fibers piercing the intestinal muscle with the blood vessels and more or less following their course. Many such fibers could be traced to the papilla where they gave off numerous branches. At the points of branching he noted that the fibers were swollen and interpreted these swellings as corresponding to the ganglia described by Oddi.

There is little doubt, therefore, that the choledochal plexus shown in figures 11 and 12 continues along the intramural course of the bile duct. Its significance lies in the fact that it may be played upon by certain extrinsic nerves⁵ and that like intrinsic plexuses in other portions of the gastrointestinal

⁵ See footnote 4.

tract (cf. Best and Taylor, '39; Evans, '26) it may be responsible for the tonus of smooth muscle — in this case the sphincters of the choledochoduodenal junction.⁶

DISCUSSION

The original program called for dissection of both the cat and the human newborn, the former to be the basis for future experimental work and the latter to provide the necessary check for a comparison with man. But the difficulty of mastering the Wharton-Sihler technique, together with the greater complexity of the biliary autonomic tracts in man and the almost unsuperable difficulty offered by the pancreas, as disclosed by preliminary dissection, has limited this study to the cat. It is desirable, however, to make whatever comparison is possible through a survey of the literature.

The classical account of Latarjet, Bonnet and Bonniot, together with such schematic drawings as that presented in figure 891 of the Spalteholz Atlas, suggest that the principal pathway to the human choledochoduodenal junction is via the plexus surrounding the bile duct. This would seem to be made up of an anterior network largely continuous with the anterior hepatic plexus — and, therefore, with the left coeliac ganglion — and a posterior net primarily connected with the posterior hepatic plexus and right coeliac ganglion. Little mention is made of the pathway along the superior pancreaticoduodenal artery. Nor would it appear that there is a single nerve in man comparable to the gastroduodenal. But it is believed

⁶ Since the experiments of Johnson and Boyden have indicated that section of the extrinsic nerves to the junction results only in retardation of the flow of bile through the ampulla — the effect of eliminating the vagal components — and that section of the splanchnics causes only slight, if any, acceleration, these authors have postulated that the tonus of the sphincter is maintained by its intrinsic plexus. Of special interest, in this connection are the observations of Sabussow and Ssuslikow ('37). They found that when both the coeliac ganglia and the vagi were eliminated in the dog, neuro-histological preparations of the gall bladder musculature still retained some undegenerated post-ganglionic fibers. These were interpreted as being post-ganglionic to the intramural ganglia of the gall bladder, i.e., they belonged to the intrinsic nerve net.

that by the use of the Wharton-Sihler method much more information can be secured regarding the distribution of fibers in the human biliary tract. The problem demands intensive investigation and the employment of refined techniques.

SUMMARY

The distribution of extrinsic nerves to the choledochoduodenal junction is mediated through two pathways (designated, respectively) the gastroduodenal nerve and the gastroduodenal plexus.

The gastroduodenal nerve is a constant bundle of fibers which parallels at a little distance the left side of the gastroduodenal artery. It arises on the ventral side of the hepatic artery by confluence of branches of the hepatic plexus (which can be traced back to both right and left coeliac ganglia) and branches of the coeliac division of the dorsal vagus. It divides into two main branches, one of which (the choledochoduodenal) terminates at the junction of the bile duct and the intestine.

The gastroduodenal plexus is a network of fibers surrounding the artery of that name and others lying between the artery and the common bile duct. It is made up of fibers coming down from the hepatic plexus and of a few recurrent fibers of the coronary nerve (a composite bundle from the left gastric plexus and the ventral vagus nerve).

These two gastroduodenal pathways give off small choledochal rami which may or may not anastomose before reaching the duct. But having reached it they form a paracholedochal plexus that descends along the duct giving off branches to an intrinsic network in the adventitia of the duct. This intramural choledochal plexus eventually enters the duodenal wall with the bile duct.

In their lower course the two pathways give off small rami to the choledochoduodenal junction. Those of the gastroduodenal plexus tend to follow the superior pancreaticoduodenal artery and its branch the common duodenal artery. Those of the choledochoduodenal nerve terminate both in the myenteric plexus adjacent to the duct and in the plexus of the duct itself.

Nerves connecting the finer choledochal and the heavier myenteric plexuses, at the point where the bile duct enters the intestinal wall, can be clearly demonstrated but there is no direct passing of one plexus into another in the sense postulated by Variot, such as would be the case if the musculature of the bile duct were to be considered merely as a continuation of the musculature of the gut wall.

The plica longitudinalis is especially well vascularized, being supplied not primarily by vessels that enter the wall with the bile duct but by numerous fine branches of the second, third, fourth, and fifth dorsal duodenal rami of the superior pancreaticoduodenal artery. These drop down from the serosa to embrace all surfaces of the ampulla and ducts.

In conclusion, it may be stated that the finding of a specific nerve to the choledochoduodenal junction and a knowledge of its components has made possible an experimental analysis of the nervous pathways regulating the evacuation of the biliary tract in the cat.

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PLATE 1

EXPLANATION OF FIGURES

5 Ventral view of cleared preparation of stomach and duodenum of an adult cat that has been injected with liquid latex (natural size). Photographed against a white background to show relation of the common bile duct (C.b.d.) to the superior pancreaticoduodenal artery and its proximal, dorsal duodenal rami (1, 2, 3, 4, 5). The duodenum has been rotated counter clockwise (as viewed from the pyloric end) and the bile duct pulled up to the reader's left to expose vessels on its deeper surface. R.g.a., right gastric artery; Cor.v., coronary vein. For further orientation, compare with text and figure 1.

6 Accurate drawing of cleared preparation of choledochoduodenal junction of cat injected with liquid latex and India ink ($\times 6$). Some orientation as in figure 5. C.b.d., common bile duct lying on first part of duodenum. Asterisk, point where duct enters duodenal wall and begins to sink obliquely toward papilla (left). 2, 3, 4, 5, second to fifth dorsal duodenal rami (cf. fig. 5). The blackest vessels lie in the serosal layer. Dilution of color indicates that the smaller branches are receding to deeper levels. Note those which are spreading out on the intramural surface of the duct. Pan., lobules of pancreas adherent to serosa. Note that the superior pancreaticoduodenal artery forks around this line of insertion. The branch above the letters is the ventral division of the artery, giving off ventral duodenal rami. That below is the dorsal division. The main stem of the superior pancreaticoduodenal artery is at the extreme right, above the letters C.b.d.

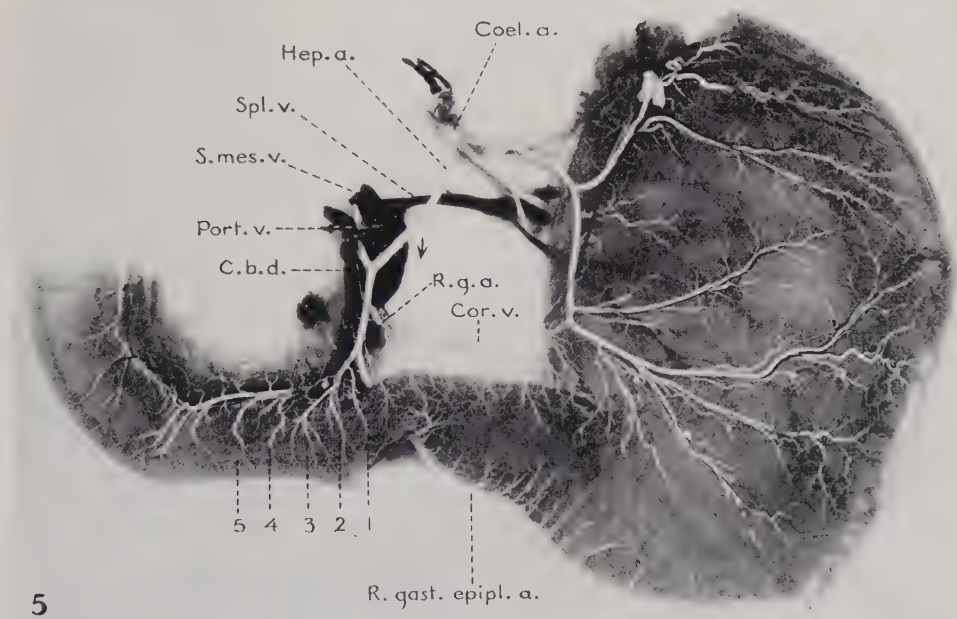


PLATE 2

EXPLANATION OF FIGURES

Photographs of fixed and stained specimens of the lesser omentum of the cat, prepared by the Wharton-Sihler technique and dissected under a binocular microscope by J. W. Schulze. Only a narrow strip of the stomach (lesser curvature) has been retained. All blood vessels, fat, and extraneous tissues have been removed by patient dissection, leaving merely the plexus of nerves. (For relation of nerves to arteries, see figs. 1 and 3). In all cases the common bile duct has been retracted and pinned to the viewer's left in order to display the fibers normally lying between the duct and the gastroduodenal artery.

7 This photograph ($\times 8/9$) reveals the enormous extent of the plexuses that follow the branches of the coeliac artery. Beginning above, note right and left splanchnic nerves (R.sp.n. and L.sp.n.) entering their respective coeliac ganglia (Gn.) and the coeliac plexus (C.pl.). Above, to the viewer's right, observe the dorsal vagus (D.v.) dividing into coeliac (C.d.) and gastric divisions (G.d.); below this, note the branches of the ventral vagus (V.v.), some of which unite with the left gastric plexus (L.g.pl.) to form the coronary nerve of the lesser curvature (C.n.). For details of fibers on side next to common bile duct (C.b.d.), see figure 8, a higher magnification of this region.

8 Enlarged photograph of choledochal side of figure 7 ($\times 1\frac{2}{3}$). Gd.n.², gastroduodenal nerve, broken during dissection. Note that it receives fibers both from the coeliac division of the dorsal vagus (Gd.n.¹) and from the coeliac plexus (C.pl.), and that it continues beneath the pylorus as the right gastroepiploic nerve (R.ge.n.). Its choledochoduodenal branch is missing. (For this branch, see Chd.n., figs. 10, 9, 4 and 3). Gd.pl.¹, gastroduodenal plexus; portion that surrounded artery of same name; Gd.pl.², second portion of gastroduodenal plexus, lying between artery and common bile duct. Both divisions (see figure) send choledochal rami to common bile duct. Their lower fibers converge to form the right gastroepiploic plexus (R.ge.pl.) which follows the artery of that name to pancreas and greater curvature of stomach. C.n., coronary nerve of lesser curvature, a continuation of fibers from the left gastric plexus and ventral vagus (fig. 7); note that it passes deep to the gastroduodenal nerve (Gd.n.²) and continues into the gastroduodenal and hepatic plexuses (Gd.pl.², H.pl.¹).

9 Photograph of another specimen ($\times 8/9$) displaying anomalous branch (An.) of ventral vagus (V.v.). This can be traced across lesser omentum to bile duct and hepatic plexus (H.pl.). Note double vagal origin of gastroduodenal nerve (Gd.n.): 1, from main stem of dorsal vagus and 2, from gastric division of that nerve. Distally it heads straight for the bile duct (C.b.d.). Pd.pl., superior pancreaticoduodenal plexus; R.ge.pl., right gastroepiploic plexus; R.g.pl., right gastric plexus. These are at a deeper level than the gastroduodenal nerve. Note that the latter forks low down, as compared with specimen in figure 10. For explanation of other legends, see figure 7.

10 Photograph of still another specimen ($\times 1\frac{2}{3}$), showing unusually high division of gastroduodenal nerve (Gd.n.) into choledochoduodenal (Chd.n.) and right gastroepiploic nerves (R.ge.n.). Note complex gastroduodenal plexus (Gd.pl.) giving off choledochal rami (Ch.r.) to the common bile duct. Observe that the gastroduodenal nerve (Gd.n.) can be traced upwards into the coeliac plexus (C.pl.) and coeliac division of the dorsal vagus (D.v.).

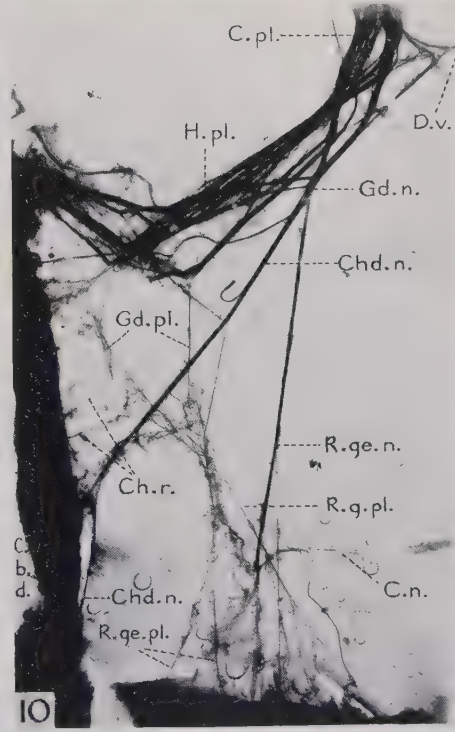
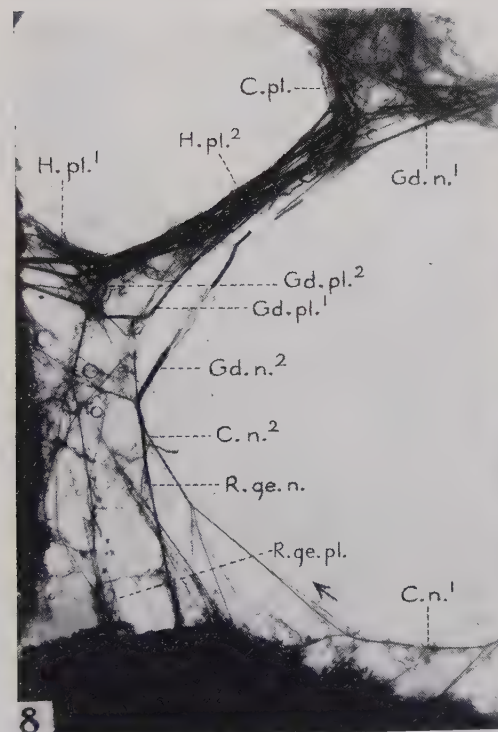
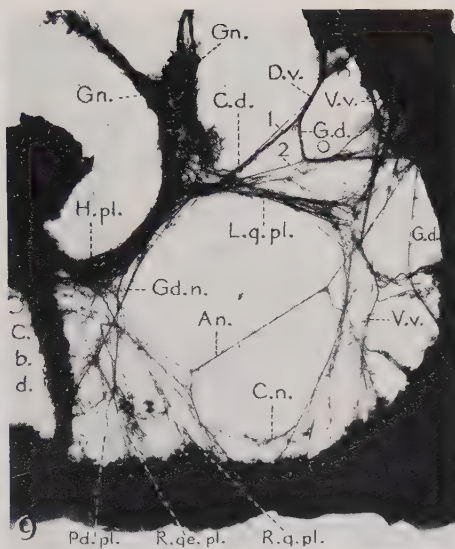
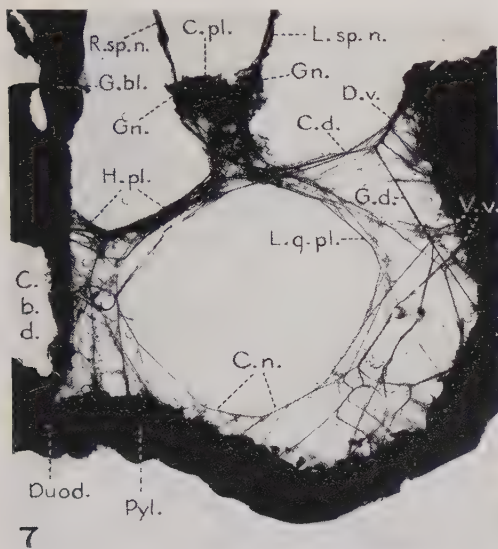
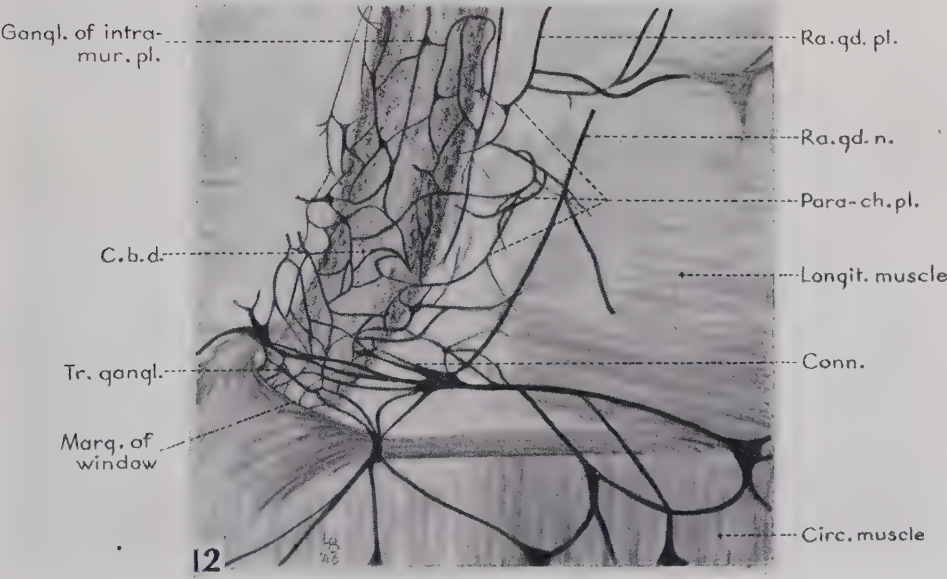
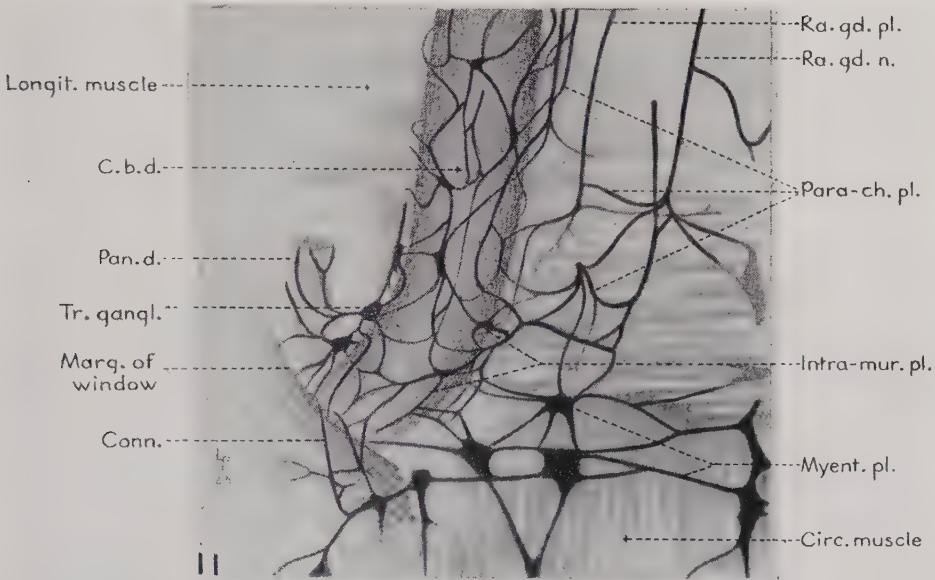


PLATE 3

EXPLANATION OF FIGURES

11 and 12 Drawings of the choledochoduodenal junction of specimens decolorized with acid alcohol ($\times 10$). The duodenal segment is spread out with the mucosal surface down. The mucosa and most of the submucosa have been scraped off to increase the translucence of the duodenal wall. The longitudinal muscle has been partially removed in order to show more clearly the myenteric plexus. In this process some of the muscle fibers around the window through which the bile duct enters the intestine have been severed, but the relations of the plexuses to the duct and window remain undisturbed. The myenteric plexus is incomplete. Some parts of it came off with the longitudinal muscle, other parts did not stain well. The intramural choledochal plexus, which lies in the fibrous coat of the bile duct, is somewhat over emphasized, being more delicate than indicated. Note that it descends with the duct through the window. Conn., small nerve trunks connecting the intramural choledochal and myenteric plexuses; Ra.gd.pl., a ramus of the gastroduodenal plexus; Ra.gd.n., a ramus of the gastroduodenal nerve; Parach.pl., extramural choledochal plexus formed by extrinsic nerves; Tr.gangl., ganglia transitional between those of the intramural choledochal and myenteric plexuses.



CHANGES INDUCED IN THE HARDERIAN GLAND OF THE GUINEA PIG BY THE INJECTION OF HYPOPHYSEAL EXTRACTS ¹

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ONE PLATE (SIX FIGURES)

During a series of studies in which exophthalmos was produced in guinea pigs by the injection of extracts of the anterior lobe of the hypophysis, it was observed that the harderian gland was hypertrophied (Smelser, '37).

The harderian gland is a large, frequently bilobed, compound, saccular gland located in the orbit of reptiles, birds and most mammals. It is rudimentary or lacking in the Anthropeoidea. Its ducts open near the nictitating membrane, but in the guinea pig, which has no nictating membrane, the ducts open into the conjunctival sac near the nasal angle. The harderian gland is apocrine in character and secretes a fatty substance. The detailed anatomy and secretory processes of this gland in the rat have been described by Bowen ('26), Davis ('29), Buschke ('33), Duthie ('34) and Grafflin ('42).

This report deals with the effects of pituitary extracts on the structure of the harderian gland in the guinea pig. The preparation of the extracts was described in detail in an earlier paper (Smelser, '38 a). In brief, they were prepared from the acetone dried anterior lobes of fresh pituitary glands which had been dissected free from the connective tissue

¹ Aided by a grant from the Francis I. and Elizabeth C. Proctor Fund. A preliminary account of these experiments appeared in *The Anatomical Record*, 1941, vol. 79 (suppl. 2), pp. 96-97.

capsule and the posterior lobe. Although most of the extracts were made from beef hypophyses, a few were prepared from sheep glands, and one from acetone dried anterior lobes of the pig gland which was prepared especially for this purpose by the Wilson Packing Company. In each instance the preparation was an aqueous alkaline extract of the dried glands and was partially purified by precipitation with 0.5 normal acid at the isoelectric point. The isoelectric soluble material was precipitated with flavianic acid, and injected as a flavianate which was partially in suspension.

The preparations from beef, sheep, and pig glands were similar in their action on the harderian and other glands. All preparations contained considerable quantities of thyrotropic, adrenotropic, and gonadotropic hormones—the latter strongly luteinizing in the dosages used. The method of preparation was such that the extracts should have possessed some growth promoting property, and very little lactogenic property. However, no tests were made for these factors.

Extracts of thymus, liver, kidney, spleen, and posterior lobe of the pituitary, prepared in the same manner as the hypophyseal extracts, were injected into other guinea pigs to provide controls. Another series of guinea pigs were injected with pregnant mares' serum, a source of gonadotropic material presumably free from adrenal or thyroid stimulating factors.

NORMAL STRUCTURE OF HARDER'S GLAND

The parenchyma of the normal harderian gland is composed of very large cells in which the nuclei are usually basally situated. The cytoplasm, which is granular, is usually visible near the nucleus, although it is obscured and displaced to a varying degree by huge intracellular lipid droplets. At some stages of the secretory cycle these droplets are nearly as large as the nucleus and completely pack the cell (figs. 1 and 3). After treatment with fixatives and reagents which dissolve lipids, the droplets appear as empty and sharply defined vacuoles. Even in such preparations the steps in the forma-

tion, growth and extrusion of these vacuoles from the cell may be readily observed. The secretion stages of these droplets have been described for the rat (Duthie, '34) and there seems to be no significant difference in the guinea pig. Considerable variation in the height of the epithelium occurs in the normal gland. In many regions the epithelium is so high that the lumen of the acinus is nearly occluded. In other acini the epithelium is lower, and the open lumina contain some debris of the coagulated secretion.

ANTERIOR PITUITARY EXTRACT INJECTIONS IN NORMAL ANIMALS

In the first group of experiments twelve young female guinea pigs were given daily subcutaneous injections of beef pituitary extract for 30 to 40 days. The daily dosage was 12.5—20 mg. of the flavianate, which is equivalent to 250

TABLE 1

The effect of anterior pituitary gland extract on the weight of the harderian glands of normal female guinea pigs.

INJECTED		UNINJECTED	
Body weight	Harderian gland	Body weight	Harderian gland
<i>gm.</i>	<i>mg.</i>	<i>gm.</i>	<i>mg.</i>
380	340	410	268
375	422	470	295
410	572	410	244
445	362	440	300
375	385	475	302
330	430	335	244
385	463	475	361
Mean	386	431	288
ϵ_M	13	12	11

to 400 mg. of the original dried powder. At autopsy the harderian glands were removed, weighed, and fixed in Zenker's or Bouin's fluid.

The injections produced a marked increase in the weight of the harderian gland (47% $P < .01$ table 1). They also produced structural modification in that most of the acini were composed of low cells which had discharged their secre-

tory substance into the acinar lumen. The earliest response to the injected pituitary extract was an obvious decrease in the number of large secretion droplets in the cells. This is perhaps best explained by assuming that the cells were activated to discharge their contained secretion. Such cells were therefore reduced in height and showed a relative increase in the amount of visible granular cytoplasm (fig. 4). In glands showing a more marked response, the epithelial cells were reduced in height from 5 or 6 to $1\frac{1}{2}$ or 2 times the diameter of the nucleus. Such cells characteristically contained many minute secretion droplets, but a few scattered ones as large as the nucleus, or even larger than any found in normal glands, were also present. The surface of the epithelium was not sharply demarcated from the lumen, indicating that some of the apical portion of the cell had been lost, as is characteristic of the apocrine type of gland. In many instances the lumina of the acini and the ducts contained considerable coagulated secretions.

The lacrimal gland, which is structurally very different from Harder's gland, was unaffected by the pituitary extract injections in this and the subsequent experiments. Thyroids, ovaries, and adrenals of the animals were definitely hypertrophied, indicating that the extracts used had contained appreciable amounts of pituitary factors affecting those glands.

EFFECT OF THYROIDECTOMY

The effect of thyroidectomy on Harder's gland was studied in fourteen uninjected young female guinea pigs (250 gm.) which had been thyroidectomized for 60 to 90 days. In each case the completeness of thyroidectomy was established at autopsy by a careful search of the cervical region for remnants of thyroid tissue.

The harderian glands of the thyroidectomized animals were 15% heavier than those in the normal controls, a difference which proved to be statistically significant ($P < .02$ table 2). There was no discernible difference between the structure of

the Harder's glands of the thyroidectomized and normal animals. The range in body weight of the animals used in this comparison was the same in the control and operated groups.

TABLE 2
Effect of thyroidectomy on the weight of Harder's gland.

THYROIDECTOMIZED (14 CASES)			NORMAL (14 CASES)	
	Body weight	Harderian gland	Body weight	Harderian gland
	gm.	mg.	gm.	mg.
Mean	491	369	482	319
ϵ_M	18	11	24	16

ANTERIOR PITUITARY EXTRACT IN THYROIDECTOMIZED ANIMALS

In another group of experiments seventeen guinea pigs were thyroidectomized and subsequently injected with the pituitary extracts in the same manner as in the first series. In each case thyroidectomy preceded the first injection by 5 to 10 days. At autopsy the harderian glands were weighed and fixed in Bouin's fluid. A very marked and consistent increase in weight of the glands was noted (table 3). The mean weight of these glands was increased from 356 mg. in the uninjected thyroidectomized animals to 546 mg. (53%, $P < .01$) under the influence of the injected pituitary extract.

The histological changes in the glands of these guinea pigs were more extreme but similar to those obtained by the injection of intact animals (figs. 2 and 5). It should be noted that not all acini responded to the same degree. While the cells of most of the acini were nearly depleted of their secretion droplets, the cells of some possessed an almost normal quota of lipid material. Such glands were composed of wide open acini with a low deeply staining epithelium, the cells of which possessed a centrally placed nucleus and a granular cytoplasm.

Measurements of the diameters of the acini of the stimulated glands gave the impression that they were either very slightly or not at all greater than those of normal ones. The increase

in the size of the lumen was gained at the expense of the epithelium.

A delicate stroma of fine collagenous connective tissue fibers forms the framework of the normal harderian gland. In the injected animals this connective tissue contained small amounts of edematous material so characteristic of the orbital fat of these animals. The amount of this infiltration, however, was

TABLE 3

The effect of anterior pituitary gland extract on the weight of the harderian glands of thyroidectomized guinea pigs.

INJECTED		UNINJECTED	
Body weight	Harderian gland	Body weight	Harderian gland
<i>gm.</i>	<i>mg.</i>	<i>gm.</i>	<i>mg.</i>
355	514	365	284
395	456	380	306
425	511	415	295
415	363	430	330
365	433	365	295
525	467	405	288
625	593	590	324
650	785	625	443
685	518	680	468
600	546	675	378
615	573	480	295
530	681	505	388
425	461	500	317
540	614	465	329
545	622	675	493
690	571	470	457
555	579		
Mean	526	501	356
ϵ_M	27	28	18

insignificant, and could have had no influence on the size of the gland.

Several of the glands from injected animals showed areas in which the epithelium had so broken down that the normal acinar structure was lost. In the cells of such areas the nuclei were frequently pycnotic. In some instances the nearly lipid-free cells were detached, and filled the lumen of the acini,

giving superficially the appearance of a solid mass (fig. 6). An apparently similar degeneration has been noted by Paulson ('37), in the harderian and "intraorbital" lacrimal glands of guinea pigs and by Lambert and Yudkin ('23), in the harderian glands of vitamin A deficient rats. Lymphocytic infiltration occurred in such areas. It is not certain that these degenerate areas are the result of the hypophyseal extract injections, for, although such conditions were not uncommon in the pituitary-treated groups, two such cases have also been found in the glands of animals treated with liver and thymus extract.

EFFECT OF CONTROL EXTRACTS AND ROLE OF THE OVARY

Except for the occurrence of the degenerate areas mentioned above, the kidney, spleen, thymus, and posterior lobe extracts were without effect on Harder's gland. These preparations, with the exception of the posterior pituitary, were given in the same dosages as those of anterior lobe pituitary extracts, and for the same length of time. Extracts of posterior pituitary tissue proved toxic and were given for shorter periods of time (15 to 20 days), and in smaller amounts.

Because the effect of the pituitary extract on the harderian glands might be due to its content of gonadotropic hormones, pregnant mare's serum (50 to 100 RU daily) was given to a group of five thyroidectomized animals for 30 days. The ovaries of these animals were enormously hypertrophied, but the harderian glands were unaffected both in weight and in structure. In the reciprocal experiment, two spayed, thyroidectomized animals were treated with pituitary extract and both showed marked harderian gland hypertrophy and structural modification which, however, were not as extreme as in most of the other cases.

The effect of an estrogen (progynon B), on the weight and structure of the harderian glands of twelve thyroidectomized female guinea pigs was determined. One hundred to 200 rat units (16.6–33.6 γ) were injected daily for 25 days. Both the body weights and the weights of the harderian glands of

the injected animals were less than those of their controls. The glands of the injected animals were normal in structure. This group of experiments indicates that the effect of the anterior pituitary preparations on the harderian glands is not mediated by the ovary.

STUDY OF FROZEN SECTIONS

All of the above observations were based on paraffin embedded sections of tissues fixed in Bouin's or Zenker's fluid and stained with Masson's trichrome stain or haematoxylin and eosin. With this technique the lipid content of the secretion droplets is lost and only empty vacuoles can be seen. In order to preserve these lipid constituents, portions of the glands of both control and beef-pituitary treated animals were also fixed in normal saline which contained 6% formalin and embedded in gelatin. Frozen sections were prepared accordingly to the technique outlined by Zwemer ('33). The sections were stained with Delafield's haematoxylin followed by Sudan III, Scharlach R., or Oil red O which were dissolved either in 70% alcohol or 70% pyridine. The Oil red O gave a deep and brilliant color reaction which was particularly satisfactory. Such sections of harderian glands of normal and thyroidectomized animals revealed cells which were an almost solid mass of fat droplets. In addition to the large intra-cellular droplets, the lumina of the acini contained secreted lipid masses, which in the paraffin sections were only faintly indicated by the small amount of coagulated material which surrounds them (fig. 5). All of the lipid material stained deeply with the various fat stains used. There was therefore no indication that more than one type of droplet was present, as was the case in the rat (Graffin, '42). The droplets in normal glands and those in glands of treated animals showed no difference in their staining reaction.

Sections were also stained with Nile blue sulphate (Smith and Mair technique) to determine whether the lipid droplets were largely neutral fat, or if appreciable quantities of free fatty acids were present. In all cases, in both treated and

control glands, pink staining droplets were found. Furthermore, the secretion filling the lumen likewise gave a pink color reaction, indicating that no, or very little, free fatty acid was present. In a very few places a suggestion of blue or lavender was found. In these places, however, the fat seemed to be associated with some superimposed blue stained secretion. (The staining reaction was controlled by observing that similar preparations of ordinary depot fat and adrenal lipids which contain free fatty acids stained a definite purple with the dye used.) The Nile blue sulphate reaction was difficult to judge in the case of small droplets, for they were embedded in a matrix of blue-stained cytoplasm, which made color differentiations difficult. Therefore, it would seem that the lipid secretion of the harderian gland consists largely of neutral fats, and that modification of the gland by the injection of anterior pituitary extracts does not change the character of the lipids in this respect.

Frequently associated with neutral fats are anisotropic compounds such as cholesterol esters. Unstained sections of glands from control and experimental animals were therefore examined with a polarizing microscope. Under crossed Nicol prisms, anisotropic compounds were visible as sharp needle-like flecks at the periphery of the secretion droplets in all cells. The anisotropic substances were present in relatively small amounts and appeared in the lumen of the acini with the other secretion products. These compounds were also found in frozen sections of fresh, unfixed glands. The anisotropic compounds reacted to the injection of pituitary extracts in the same manner as did the isotropic ones.

The Schultze test for cholesterol was applied to sections of a number of glands from normal and from injected animals, all with negative results. Since a positive reaction to this test was readily obtained with sections of the adrenal cortex, it seems that either there is extremely little cholesterol present in the harderian gland, or that the anisotropic compounds described above are some substance other than cholesterol.

QUANTITATIVE DETERMINATION OF THE FAT AND WATER
COMPONENTS

Study of paraffin preparations revealed quite a different picture from that seen in frozen sections of the Harder's glands from the pituitary-extract treated animals. The paraffin sections suggested that the lipid content of glands from treated animals was much less than normal, whereas frozen sections of such glands showed that large amounts of fat were present. However, in these sections it was extremely difficult to determine the cells' apical boundary and if the lipids were within the epithelium or had been extruded into the lumen, for such glands when stained with Sudan III or Oil red O appear to be an almost solid mass of lipid droplets.

Because of this apparent discrepancy between the amount of fat noted in paraffin and frozen sections, the water and lipid content of harderian glands of treated and untreated animals was determined. Sixteen thyroidectomized guinea pigs were used. Ten of these were injected with anterior pituitary extract as in the preceding experiment, the six control animals were uninjected. At autopsy the glands were removed from the orbit, finely minced with scissors and placed in a weighing bottle. The tissue was distributed over the inside of the bottle in as thin a film as possible. The water content was determined by drying to constant weight at 80°C. over phosphorus pentoxide in a moderate vacuum. The fat contained in the dry residue was removed by five to eight extractions with ether. The ether was poured from the first vial, without a loss of tissue residue, into a second weighing bottle, where it was evaporated and the ether soluble material weighed. The amount of lipid determined in this manner agreed closely with the amount of decrease in weight of the dry tissue after the ether extraction process. Additional extractions with ether did not increase the yield of fat, thus indicating that the extraction was complete.

The data obtained by this procedure (table 4), indicate that the anterior pituitary extract had caused an hypertrophy in which water, lipid, and residual constituents of the gland

retained approximately normal proportions. The somewhat smaller proportion of lipids in the hypertrophied glands, which is more evident if the data are analyzed from the standpoint of per cent increase may be the result of an increased rate in the discharge of the secretory product.

TABLE 4

The effect of anterior pituitary gland extract on the water and lipid (ether soluble) content of the harderian glands of thyroidectomized guinea pigs.

	INJECTED				UNINJECTED			
	Total	Water	Lipid	Residue	Total	Water	Lipid	Residue
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
	511	330	123	58	344	216	85	43
	456	312	82	62	317	198	77	42
	418	241	126	51	329	182	105	42
	615	372	165	78	383	208	127	48
	788	498	186	104	493	298	128	67
	623	381	168	74	492	302	125	65
	571	405	85	81				
	652	419	139	94				
	609	402	124	83				
	578	377	129	72				
Mean	582	374	133	76	393	234	108	51
ϵ_M	33	22	12	5	33	16	9	5
Proportions, per cent								
	100	64	23	13	100	60	27	13
<i>Comparison of the data from injected and uninjected animals.</i>								
				Total				Residue
Increase				189 mg.	140 mg.	25 mg.		25 mg.
Probability of								
random sampling				< .01	< .01	.20		< .01

DISCUSSION

The above data show that extracts of anterior hypophyseal tissue contain some factor which markedly affects the size and structure of the harderian gland. Because similarly prepared extracts of other tissues do not have this property it is evident that the factor is a characteristic of the anterior lobe of the pituitary gland. At present no particular relation-

ship can be suggested between the harderian gland and the endocrine system. However, it should be no more surprising to find that the pituitary gland exercises a control over a conjunctival gland, than it was to find that it stimulated the esophageal crop glands of the pigeon or possibly even the mammary glands.

The function of Harder's gland is not known. Its secretion certainly aids in the lubrication of the cornea and lids, but this function is cared for by the lacrimal gland. Collins ('30) suggests that mucins contained in the harderian secretion, protect the corneal epithelium from keratinization. However, removal of Harder's gland in rabbits (Wessely, '28) rats (Figge and Salomon, '42), or guinea pigs (Smelser, '43) had no observed deleterious effects.

The relationship of the substance in pituitary extracts which activates Harder's gland to any of the anterior hypophyseal hormones is not clear in the present experiments. It is worth pointing out that the preparations which activated Harder's gland also had marked thyrotropic and gonadotropic potencies. Assays, utilizing the sensitive 4-day-old rat as a test object, indicated that only about one unit of adrenotropic hormone was administered per day. That the gonadotropic factors contained in the extract were responsible for the reaction of the harderian gland is somewhat doubtful in view of the failure of pregnant mares' serum to provoke any response. It is, of course, recognized that the gonadotrope in pregnant mares' serum differs in other respects from gonadotropic extracts of pituitary gland origin. Since the harderian gland hypertrophy was obtained by the injection of pituitary extract into spayed animals, and because the injection of an estrogenic hormone was without effect on its size or structure, it may be concluded that the ovary is not directly involved in this reaction. Similarly, since the pituitary extract was extremely effective in animals which had been thyroidectomized, it seems certain that the changes described are not mediated by the thyroid gland. Indeed, the histological modification of the harderian gland appeared to be more extreme in the thyroidectomized

animals. Ablation of the thyroid alone is shown to cause a slight, though definite, hypertrophy of Harder's gland, an effect which may be correlated with the reported decrease in harderian gland weight of hyperthyroid guinea pigs (Smelser, '38 b).

The interpretation of the histological appearance of the activated gland must be made with reference to the analysis of the glands as well as to a study of the frozen and paraffin sections. Since the frozen sections show that the Harder's glands of the injected animals contain much more fat than is indicated in paraffin preparations it seems probable that most of the lipid material is located in the apical portion of the cells and in the lumina of the acini. The quantity of lipoidal substances found by chemical analysis in harderian glands of both control and experimental animals is in harmony with the study of frozen sections. The data obtained by analysis of the glands of course do not permit distinction between secreted materials in the lumen of the acinus and the intra-cellular components.

The evidence at our disposal indicates that the injection of pituitary extract causes a marked hypertrophy of Harder's gland in both normal and thyroidectomized animals. This hypertrophy is accompanied by pronounced structural changes in the epithelium indicating an increase in discharge of the lipid droplets. The presence of many small secretory droplets suggests that the cells are actively replacing their secretion product.

The reaction of Harder's gland to injection of pituitary extracts suggests that other exocrine glands may be under some degree of pituitary control. Preliminary data on hand indicate that similar changes occur in the sebaceous glands of the guinea pig.

SUMMARY

1. Injection of extracts of anterior pituitary glands in guinea pigs causes a marked hypertrophy of the harderian gland. There is an extensive discharge of the lipid secretion

from cells of the secretory epithelium which appear also to be actively elaborating new secretion as indicated by the presence of many small secretory droplets.

2. These effects are readily produced in thyroidectomized animals, and it may be that thyroidectomy actually enhances the response.

3. The secretion product of Harder's gland is mainly neutral fat, although some anisotropic compounds are included and some non-lipid substances in both normal and activated glands.

4. The reactions described have so far been induced only by extracts of the anterior pituitary gland, but no definite association of the active principle with any of the commonly accepted pituitary hormones is attempted.

I wish to express my appreciation and my thanks to Miss Victoria Ozanics for her able assistance throughout this study.

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PLATE 1

EXPLANATION OF FIGURES

1 Harderian gland of a thyroidectomized guinea pig. This gland shows the characteristic normal structure. $\times 104$.

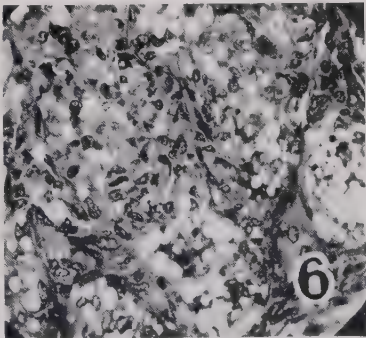
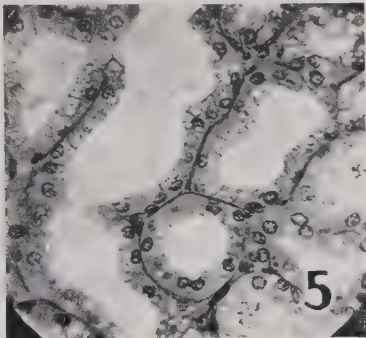
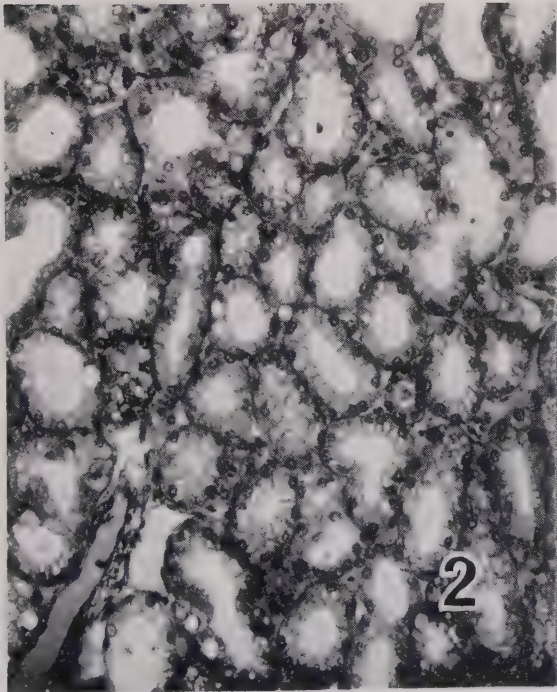
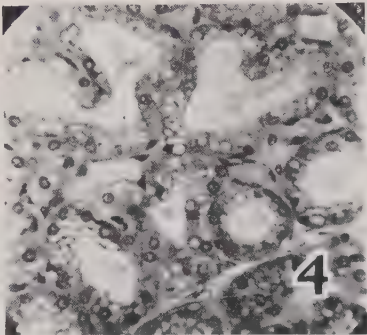
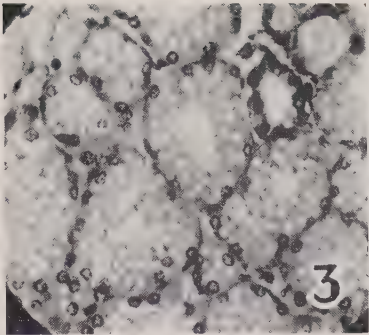
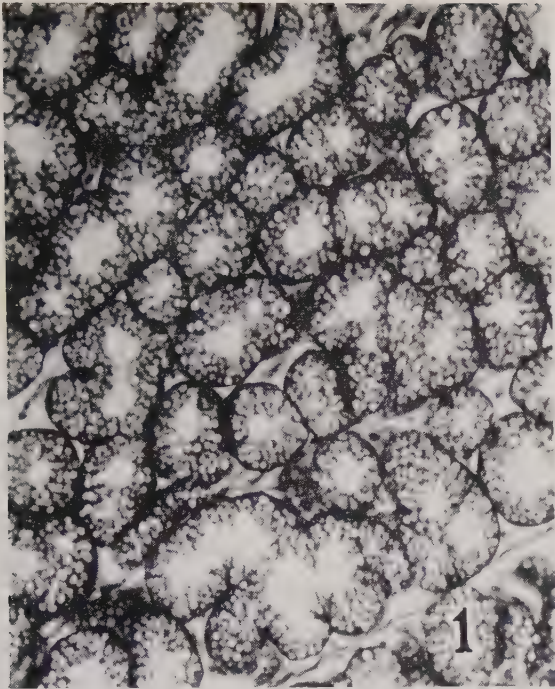
2 Section of a harderian gland of a thyroidectomized guinea pig which had been injected with an anterior pituitary extract. There has been an extensive discharge of the large secretion droplets but numerous small ones are being formed. A few very large droplets may be seen. $\times 104$.

3 Harderian gland of a normal guinea pig showing the characteristic, large, highly vacuolated cells. The vacuoles are spaces which contained lipids. $\times 234$.

4 Harder's gland of a normal guinea pig injected with an extract of the anterior lobe of the hypophysis. $\times 234$.

5 The harderian gland of a thyroidectomized guinea pig injected with an hypophyseal extract. Note the decrease in size and number of vacuoles and the relative increase in cytoplasm. $\times 234$.

6 A section of Harder's gland of a thyroidectomized guinea pig treated with anterior pituitary extract, showing an area in which the lumina of the acini are filled with cellular debris. The epithelium is atrophic in some places. $\times 234$.



THE NORMAL DEVELOPMENT OF TRITURUS PYRRHOGASTER^{1, 2}

PRISCILLA L. ANDERSON³

FOUR PLATES (THIRTY-SIX FIGURES)

Because of the use of *Triturus pyrrhogaster*, the Japanese fire-bellied salamander, in experimental embryology, a study of its normal development may prove valuable. The following tables and illustrations are offered with the purpose of correlating internal development with the external appearance of the embryo up to the age of 6 weeks after fertilization. Normal studies have already been made on other amphibians, including *Rana sylvatica* (Pollister and Moore, '37), *Rana pipiens* (Shumway, '40), *Amblystoma punctatum* (Harrison stages, unpublished), *Triturus viridescens* (Gage, 1891; Jordan, 1893), and *Molge vulgaris* (L. Glaesner, '25). These works pertain largely to external development, while the present paper will attempt to discuss internal development along with the external.

METHODS

Eggs for this study were obtained from animals procured from the Nippon Goldfish Company in San Francisco. Ovulation was induced by means of pituitary injections and implantations after the methods of Rugh ('34, '35). Artificial fertilization was not necessary, since the females pick up

¹ Contributions from the Department of Zoology, Smith College, no. 197.

² From a thesis submitted in partial fulfillment of the requirements for the degree of Master of Arts, Smith College, May 1936.

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spermatophores and carry them until such time as eggs are laid. The eggs are then fertilized in the cloaca just before they are laid (Jordan, 1893). Eggs for the timing of stages were kept in small dishes in tap water at an average temperature of 18°C. Due to lack of apparatus for perfect control, there were occasional, comparatively slight, fluctuations in temperature, through a range of not more than ± 1 degree.

Material for sections was fixed in Smith's Fluid, dehydrated in dioxan, and imbedded in paraffin. Sections were cut at 15, 20, and 25 micra. The outlines of all drawings were made with a camera lucida at a magnification of $21\times$. All photomicrographs of sections were made at a magnification of $33.5\times$.

OBSERVATIONS

All observations have been summarized in the following tables and plates. In table 1 the numbers used on his plates by Harrison to designate stages of development in *Amblystoma punctatum* have been indicated for the sake of comparison, although in several cases a close correlation between the two different salamanders is difficult to achieve.

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TABLE 1
Stages studied at 18°C.

STAGES T. PYRRHO- GASTER	HARRISON STAGES A. PUNCTATUM	AGE	COMMENTS
I	1	Newly laid	
II	2	15.5 hrs.	First cleavage incomplete
III		20 hrs.	Second cleavage incomplete
IV	3	21 $\frac{3}{4}$ hrs.	Four cell
V		23 $\frac{1}{2}$ hrs.	Third cleavage incomplete
VI	4	25 hrs.	Eight cell
VII		26 hrs.	Fourth cleavage incomplete
VIII	5	27 hrs.	Sixteen cells
IX	6	31 hrs.	Thirty-two cells
X	9	2 days	Middle blastula
XI	10	4 days	Early gastrula; dorsal lip of blastopore
XII	11 ⁺	4 $\frac{1}{2}$ days	Middle gastrula; blastopore large
XIII	12	5 days	Late gastrula; yolk plug
XIV	13	6 days	Blastopore closed to a slit
XV	15	7 days	Early neural plate
XVI	16 ⁻	7-7 $\frac{1}{2}$ days	Later neural plate
XVII	17	7 $\frac{1}{2}$ days	Late neural folds
XVIII	19	8 days	Neural folds fusing
XIX	20	8 $\frac{1}{2}$ days	Neural tube just formed
XX	27	10 days	Head formed
XXI	31	13 days	Early tail-bud
XXII	35	15 days	External gills beginning to form
XXIII	37	17 days	Fore limb-bud
XXIV	40 ⁻	3 weeks	Two digits on fore limb-bud
XXV	46 ⁻	6 weeks	Hind limb-bud

TABLE 2
Comparison of stages.

STAGE	AGE	LENGTH	EXTERNAL APPEARANCE	INTERNAL APPEARANCE
I	Newly laid	2 mm.	Light area at animal pole. Clear spot in light area. Dark dot in center of clear spot.	
II	15.5 hrs.	2 mm.	First cleavage plane partly formed; not evenly spaced in pigmented area.	
III	20 hrs.	2 mm.	Second cleavage plane on animal pole at right angles to first.	
IV	21.75 hrs.	2 mm.	Four blastomeres. Animal pole of each pigmented, and lower filled with yolk.	
V	23.5 hrs.	2 mm.	Third cleavage beginning to pinch off top center of each of cells to form four micromeres.	
VI	25 hrs.	2 mm.	Eight blastomeres: 4 pigmented micromeres on top, and 4 yolk-filled macromeres beneath.	Small blastocoel.
VII	26 hrs.	2 mm.	Each of 4 micromeres dividing in half to form 12 cells.	
VIII	27 hrs.	2 mm.	4 macro- as well as 4 micromeres now divided in half, forming 16 cells.	
IX	31 hrs.	2 mm.	32 cells: upper 16 pigmented, lower 16 not.	Blastocoel with single layer of cells above, and large yolk-filled cells below.
X	2 day	2 mm.	Middle blastula: very small pigmented cells at animal pole. Unpigmented and slightly larger at vegetal pole.	Yolk-filled cells below blastocoel smaller than previous stage.
XI	4 day	2 mm.	Dorsal lip of blastopore visible. Cells within curve of dorsal lip somewhat larger and unpigmented.	Dorsal lip invaginated enough so that very thin archenteron visible.
XII	4.5 day	2 mm.	Blastopore large and completed. Dorsal lip more accentuated than ventral.	Archenteron slightly larger than blastocoel and with 2-layered roof: outer layer ectoderm, inner one of notochordal plate plus mesoderm.
XIII	5 day	2 mm.	Yolk plug small and slightly compressed laterally.	Archenteron large. Small remainder of blastocoel at anterior end extending through about one-fifth of egg.

TABLE 2 (Continued)

STAGE	AGE	LENGTH	EXTERNAL APPEARANCE	DIGESTIVE TRACT	MESODERM	NERVOUS SYSTEM
XIV	6 day	2 mm.	Blastopore closed to a slit. Faint indication of neural groove.	In mid-region lumen about equal in size to yolk mass. Bounded dorsally by plate of notochordal cells. Endodermal roof incomplete.	Double layer of thin cells reaching in mid-region $\frac{3}{4}$ of distance to mid-ventral line.	
XV	7 day	2.14 mm.	Embryo no longer spherical. Neural folds appearing.	Lumen very large at anterior end. Smaller in mid-region.	Extending ventrally as far as neural folds at extreme anterior end. Meeting in mid-ventral line in mid-region and posteriorly. Cavity of mesomere visible.	N. folds low and widely separated anteriorly; closer in mid-region flattening posteriorly.
XVI	7-7 $\frac{1}{2}$ day	2.14 mm.	Gill plate barely distinguishable.			Neural folds closer; approaching each other in anterior region.
XVII	7 $\frac{1}{2}$ day	2.19 mm.	Neural folds close. Gill plate clearly marked.	Lumen large at anterior end, small in mid-region.	Meeting in mid-ventral line about $\frac{1}{3}$ of length from anterior end and posteriorly. Cavity of epimere visible.	Neural folds nearly touching $\frac{1}{3}$ of distance from anterior end. Touching at anterior end of neural plate.

TABLE 2 (Continued)

STAGE	AGE	LENGTH	EXTERNAL APPEARANCE	DIGESTIVE TRACT	MESODERM	NERVOUS SYSTEM	EYES	EARS	NOSE	HEART	KIDNEY	STAGE
XVIII	8 day	2.61 mm.	Gill plate more definite. Somites faintly visible.	Large pharynx at anterior end.	Somites well-formed in anterior half of body. Posterior half an undifferentiated sheet of mesoderm.	Neural folds touching entire length; thickened, and cavity enlarged at anterior end.						XVIII
XIX	8½ day	2.76 mm.	Somites and gill plate more prominent.	Large anterior pharynx. Very small liver diverticulum.	Somites well-formed.	Neural tube formed. Brain anterior to pharynx.	Optic vesicles very small.					XIX
XX	10 day	2.90 mm.	Well defined head. Optic vesicle, otocyst, gill plate, somites visible.	Large anterior pharynx.	Cavity of fore-brain large.		Optic vesicles clearly defined. Optic stalk beginning.					XX
XXI	13 day	3.85 mm.	Head and tail-bud well marked. Olfactory pit forming.	Liver diverticulum larger.	Epimere, mesomere, and hypomere separated.	Large cavity of hind-brain forming. Roof very thin.	Cup-shape of optic vesicles beginning.	Otocysts invaginating, lateral to hind-brain.	Thickening of small area of ectoderm ventral to eye.		One or two pronephric tubules in nephrotome each side.	XXI
XXII	15 day	5.47 mm.	Tail-bud longer. Body elongated and thin. Balancer appearing. Gill plate divided into three arches.	Liver diverticulum elongated.	Brain large. Cavity of hind-brain greater. Cranial nerves V, VII, VIII. (Buds of lateral line sense organs.)	Optic cup and lens formed.	Otocysts isolated. Auditory nerve.		Invagination of thickened ectoderm ventral to eye.	Forming ventral to pharynx.	2 tubules, pronephric duct complete into cloaca, and pronephric funnel each side.	XXII

TABLE 2 (Continued)

STAGE	AGE	LENGTH	EXTERNAL APPEARANCE	DIGESTIVE TRACT	MESODERM	NERVOUS SYSTEM	EYES	EARS	NOSE	HEART	KIDNEY	SKELETON	ETC.	STAGE
XXIII	17 days	7.47 mm.	Balancer and 3 gills evaginating. Fore limb-bud area visible. Pigment appearing. Dorsal fin formed.	Mouth beginning.		Hind-brain cavity very large and thin-roofed.	Optic cup more rounded. Lens well formed.	Larger, with endolymphatic duct.	Olfactory pit deep.	Blood cells distinguishable in heart and in blood vessels.	Pronephric tubules, duct, and 2 funnels each side. Pronephric glomeri visible.			XXIII
XXIV	3 weeks	10.47 mm.	Balancer completed. Gills many branches. Pigment increased. Fore limb with 2 digits. Heart visible through skin.	Thick-walled stomach and liver clearly defined. Still solid yolk mass posterior to liver. Small rudiments of teeth on jaws.	Chewing muscles prominent. Yolk gone from muscle tissue.	Regions of brain recognizable. Optic and olfactory nerves.	Lens, retina with layer of rods and cones, choroid coat.	Otocyst divided into small lateral and large medial chambers.	Large olfactory capsule attached to olfactory lobe of brain.	Anterior to liver.	Many pronephric tubules. Also funnels and glomeri, apparently 2 in number.	Cartilage around notochord, ventral to fore-brain, in both jaws and in gill arches.	A few mucous glands in skin.	XXIV
XXV	6 weeks	14.28 mm.	Much pigmentation. Gills and fore limb complete. Balancer gone. Hind limb-bud visible. Mouth open. Heart and intestine visible through skin.	Teeth on both jaws. Tongue in mouth. Liver containing gall bladder and bile duct. Intestine full length of body, coiled in anterior region, and with much yolk still present in walls. Stomach J-shaped with lining in high rugae.	Some eye musculature.	All regions were differentiated. Dorsal ganglia of spinal nerves between somites.		Three chambers.	Internal nares open.	Ventral to gills.	Pronephric ducts dorsal to digestive tract.	Cartilage ventral and lateral to brain. Hyoid cartilages, gill arches, and both jaws. Cartilage in fore limb and around ears. Narrow bar of bone in lower jaw for attachment of teeth.	Many mucous glands in skin.	XXV

PLATE 1

EXPLANATION OF FIGURES

FIG.	STAGE	AGE	LENGTH	REMARKS
1	II	15½ hrs.	2 mm.	Animal pole. First cleavage incomplete.
2	VII	26 hrs.	2 mm.	Animal pole. Fourth cleavage beginning.
3	XII	4½ days	2 mm.	Middle gastrula. Blastopore complete but large.
4	XIII	5 days	2 mm.	Late gastrula showing yolk plug.
5	XIV	6 days	2 mm.	Blastopore closed to a slit. Neural groove just appearing.
6	XV	7 days	2.1 mm.	Early neural plate with neural folds.
7	XVI	7-7½ days	2.1 mm.	Neural folds approaching each other. Gill plate forming.
8	XIX	8½ days	2.8 mm.	5-6 muscle somites. Neural folds fused to form neural tube.
9	XX	10 days	2.9 mm.	Lateral aspect. Optic vesicle, otocyst, and gill plate.
10	XXI	13 days	3.9 mm.	Lateral aspect. Olfactory pit, optic cups, gill plate, early tail-bud.
11	XXI	13 days	3.9 mm.	Dorsal aspect. Gill plate, hind-brain.
12	XXII	15 days	5.5 mm.	Dorsal aspect. Balancer, gill plate, ventricle of hind-brain.
13	XXIII	17 days	7.5 mm.	Dorsal aspect. Balancer, external gills, ventricle of hind-brain, fore limb-bud, dorsal fin.
14	XXIII	17 days	7.5 mm.	Lateral aspect. Optic cup, otocyst, balancer, external gills, fore limb-bud, anus, dorsal fin.
15	XXIV	3 wks	10½ mm.	Lateral aspect. Balancer, gills, anus, fore limb-bud with 2 digits.
16	XXV	6 wks.	14.3 mm.	Ventral aspect. Open mouth and hind limb-bud. Heart, eyes, and coiled intestine faintly visible through skin.

NORMAL DEVELOPMENT T. PYRRHOGASTER

FRISCILLA L. ANDERSON

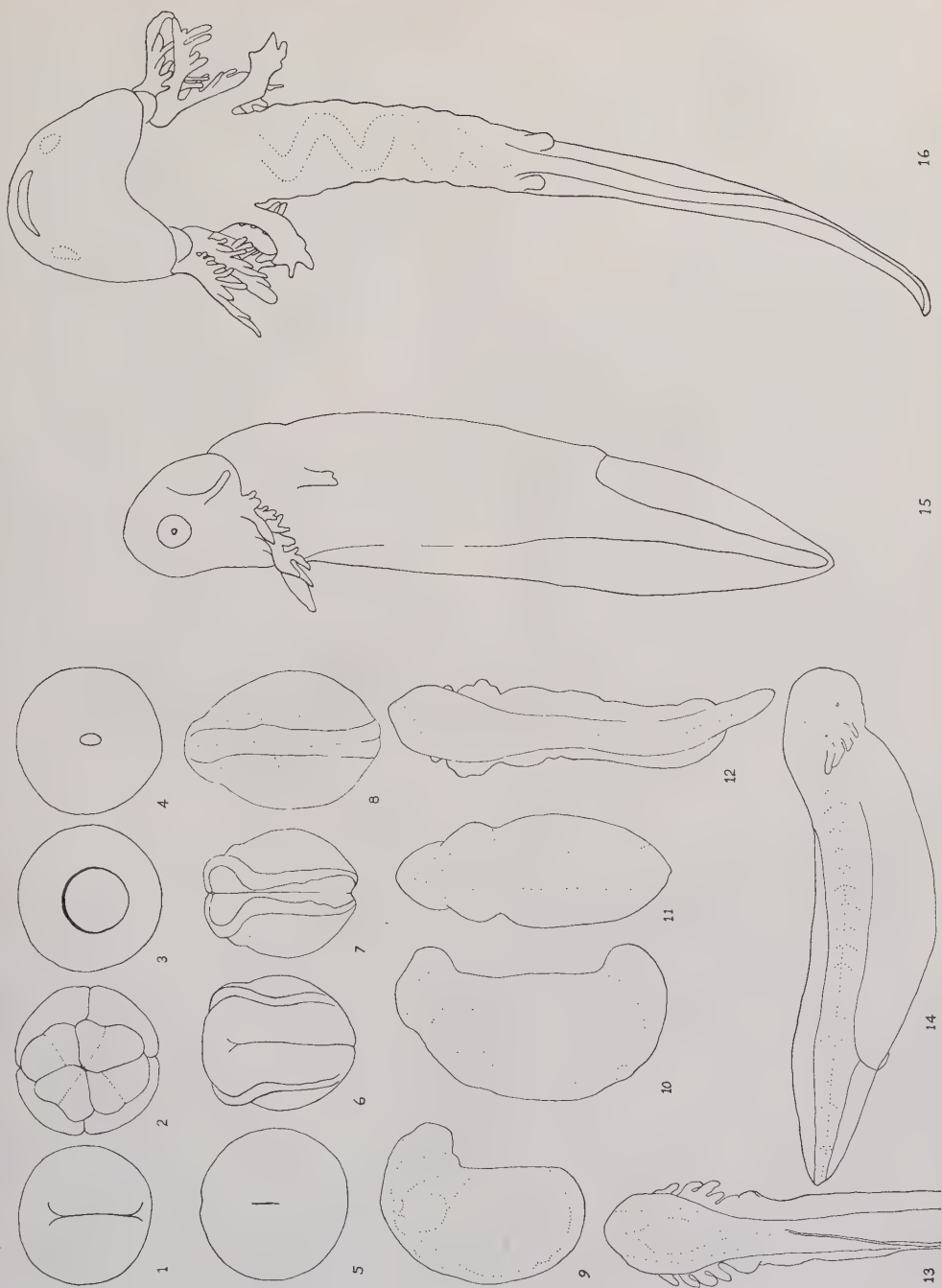


PLATE 2

EXPLANATION OF FIGURES

FIG.	STAGE	AGE	REMARKS
17	VI	25 hrs.	Section of 8-cell stage showing blastocoel and chorion.
18	IX	31 hrs.	Section of 32-cell stage.
19	XI	4 days	Early gastrula. Sagittal section showing invagination of dorsal lip of blastopore.
20	XIII (fig. 4)	5 days	Late gastrula. Sagittal section showing yolk plug, large archenteron with notochordal plate above it, and small remnant of blastocoel.
21	XIV (fig. 5)	6 days	Blastopore closed to a slit. Cross section through mid-region. Endoderm incomplete ventral to notochordal plate.

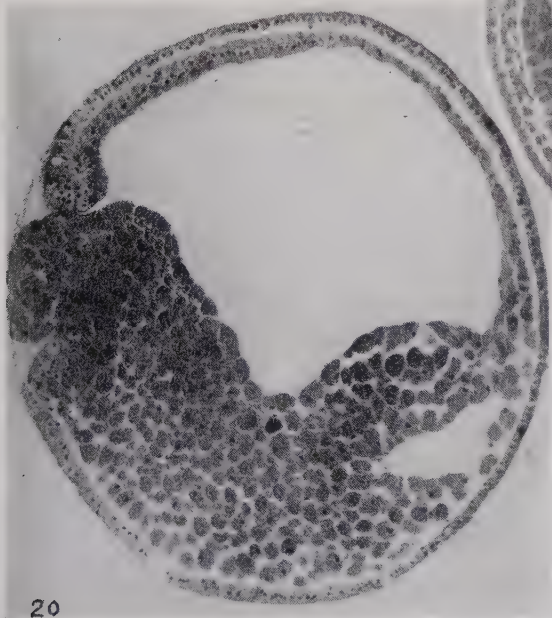
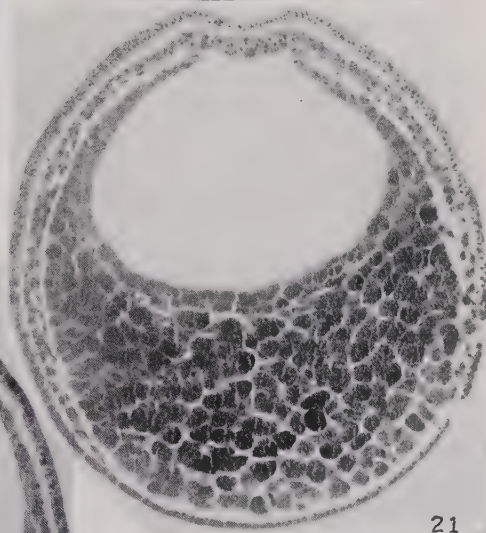
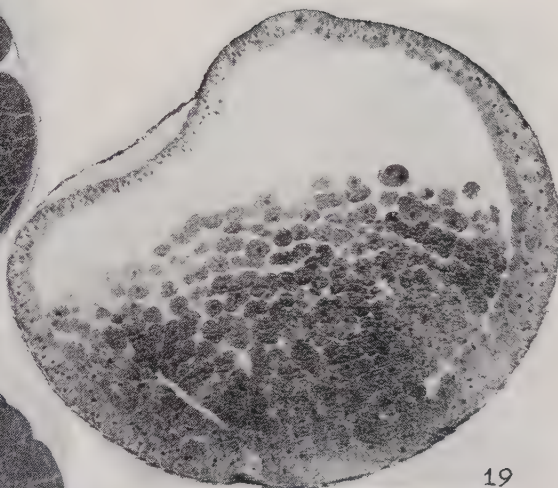
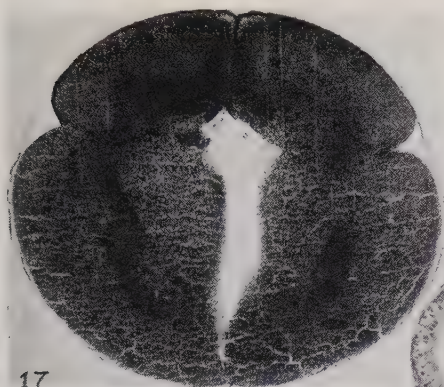
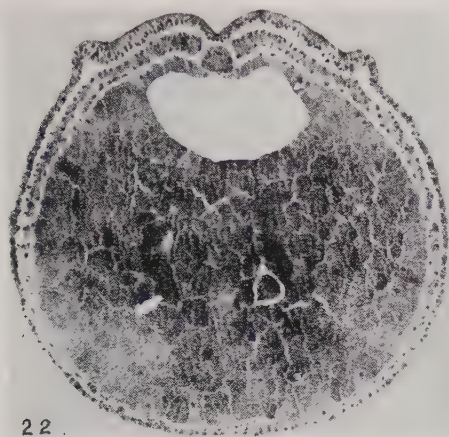


PLATE 3

EXPLANATION OF FIGURES

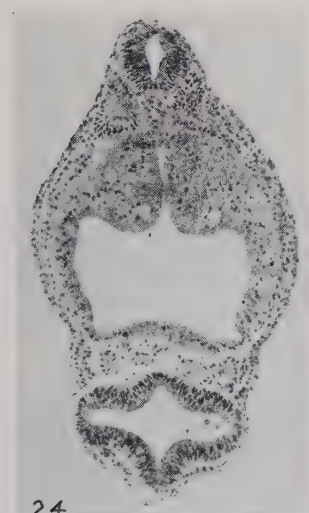
FIG.	STAGE	AGE	REMARKS
22	XV (fig. 6)	7 days	Early neural plate. Cross section through mid-region. Mesoderm meeting in mid-ventral line. Endodermal roof of archenteron nearly complete.
23	XVIII	8 days	Cross section through anterior end. Neural folds nearly fused, notochord, large pharynx, well-formed somites.
24	XX (fig. 9)	10 days	Cross section through anterior end. Large pharynx with fore-brain and optic vesicles ventral to it.
25	XXI (figs. 10 & 11)	13 days	Cross section through head. Otocysts lateral to hind-brain. Optic vesicles evaginating from fore-brain.
26	XXI (figs. 10 & 11)	13 days	Cross section through same embryo as figure 25, about mid-region. Liver diverticulum in ventral part of yolk mass, small digestive tube in dorsal part. Nephrotome a tubular mass lateral to somites.
27	XXII (fig. 12)	15 days	Cross section about one-fourth distance from anterior end. Otocysts lateral to hind-brain. Gill arches lateral to pharynx and heart ventral to it.
28	XXII (fig. 12)	15 days	Cross section posterior to liver. Pronephric funnels and tubules in mesoderm lateral to small digestive tube.
29	XXIII (figs. 13 & 14)	17 days	Cross section through head. Eye with lens lateral to fore-brain, and anterior end of digestive endoderm ventral to it.
30	XXIII (figs. 13 & 14)	17 days	Cross section one-fourth of distance from anterior end. Mid-ventral blood vessel, small pronephric glomus each side of dorsal aorta, and pronephric funnel visible on right side.



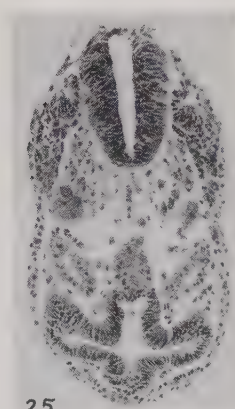
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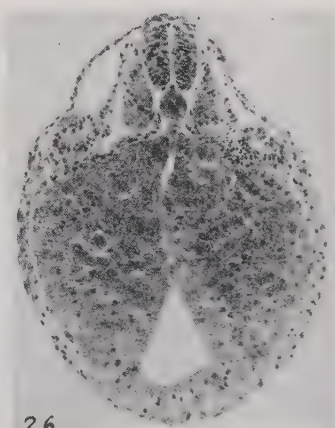
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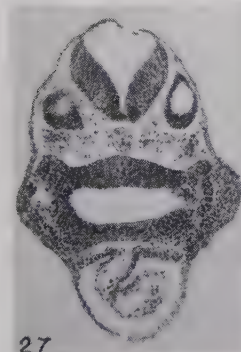
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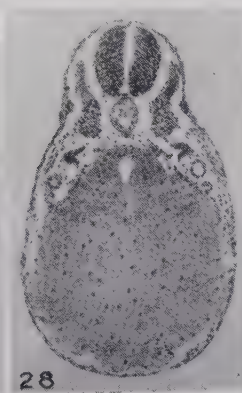
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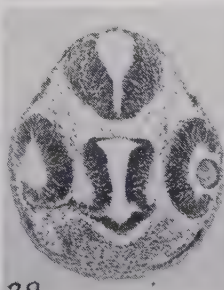
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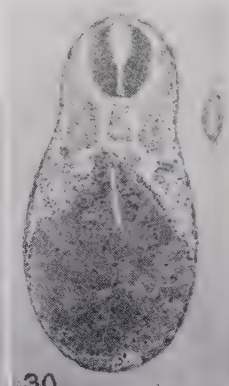
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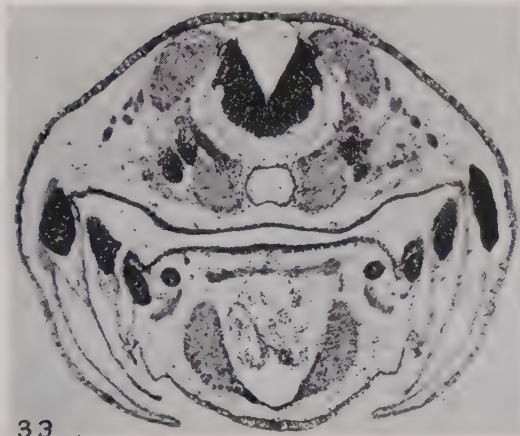


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PLATE 4

EXPLANATION OF FIGURES

FIG.	STAGE	AGE	REMARKS
31	XXV (fig. 16)	6 wks.	Cross section through head. Well-developed eye with lens, retina, and choroid coat. Cartilage appearing ventro-lateral to brain. Tongue in mouth.
32	XXV (fig. 16)	6 wks.	Cross section through ear region. Three chambers of ear and auditory nerve.
33	XXV (fig. 16)	6 wks.	Cross section through gill arches, heart, and myelencephalon.
34	XXV (fig. 16)	6 wks.	Cross section anterior to forelimb. Liver ventral to stomach, pronephric glomi from ventral side of aorta dorsal to stomach, pronephric tubules lateral to glomi.
35	XXV (fig. 16)	6 wks.	Cross section through forelimb region. Gall bladder in liver; pronephric duct dorsal to stomach.
36	XXV (fig. 16)	6 wks.	Cross section just posterior to attachment of forelimb. showing histological differentiation in coils of intestine. Most dorsal and dextral of coils is small posterior end of stomach.



THE METHOD OF REPAIR IN EPITHELIAL WOUNDS OF THE CORNEA ¹

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TWO FIGURES

INTRODUCTION

The events responsible for the epithelial healing of wounds have been identified variously in the past. Cell division, cell migration, connective-tissue metaplasia, and different combinations of these factors, have all had their proponents at one time or another. But the principal question has centered on whether epithelial migration or cell division is primary.

The culturing of epithelium by Loeb ('02) and many subsequent workers has proved that these cells are capable of amoeboid activity. Other investigations (and certain collateral proofs) point toward the correctness of Barfurth's (1891) pioneering belief that wounds are first epithelized by the active migration of the surrounding epithelial cells; mitosis, which Fraisse (1885) thought was all sufficient, occurs later in order to restore the depletion. Incontrovertible proof of this sequence of a primary epithelial mobilization and movement has been furnished by Arey ('32) for the healing of small skin wounds in fishes and amphibia. Much information on the events of wound healing and a comprehensive bibliography covering these topics have been presented in a review (Arey, '36).

The same controversy over the rôles of cell migration and cell division has confused the specific problem of corneal repair. The earlier literature on this subject has been reviewed

¹ Contribution from the Anatomical Laboratory, no. 390.

by Jusélius ('10) and Salzer ('12). In more recent years the primacy of cell division has been argued by Jusélius ('10), and Törö ('32). Initial cell migration and subsequent cell proliferation have been upheld by Salzer ('11) on the rabbit, and by Gurwitsch ('26) on the frog.

The present experiments were carried out to ascertain the relation of mitosis and cell migration to the healing of simple epithelial defects in the mammalian cornea. In particular was it desired to establish some quantitative details as to the time and frequency of cell division with respect to the events of healing. Such data had not previously been placed on record.

MATERIAL AND METHODS

After some preliminary trials proved that use of the dog's cornea introduces certain practical difficulties, attention was directed to the white rat which was then employed with entire success. All of these animals were sexually mature, but the series was too small to test such factors as age or sex in the reparative process. The experimentation was carried out in the summer, autumn and winter, yet the temperature and other environmental factors were maintained at a fairly constant level.

It was desired that the wounds be trench-like, with fairly steep sides, and approximately equal in size. This type of lesion was produced by a whirling disk of sand paper, such as a dentist uses in polishing fillings, actuated by a dental engine. These discs have a thin, flat edge and, when revolving rapidly, scrape out a clean trench. In successful operations the substantia propria is not harmed. When first made, the cut was usually about 0.3 mm. across, but epithelial tension produced gaping to a width of about 0.5 mm. All the wounds were 3 mm. in length.

Healing proceeded rapidly and infection was not a matter of practical concern. In fact, even after a few hours the wound usually could not be identified grossly. Consequently, in order to facilitate later orientation, the cut was made along an imaginary line crossing the eyeball from the outer to the

inner canthus, with the eye held in a neutral position. Later, just before the animal was sacrificed, two tiny stitches were placed in the conjunctiva near each canthus. In the absence of these precautions, proper orientation for sectioning was uncertain and the results were often not uniform or favorable.

At intervals of 6 hours to 20 days after the wound had been made, the rats were killed² and the enucleated eyeballs dropped into Zenker's fixing fluid. When the eyeballs had hardened sufficiently in subsequent alcoholic treatment each cornea was dissected free. Using the stitches as landmarks, the cornea was trimmed to a rectangle whose long sides ran parallel with the rectilinear wound. The latter occupied an approximately central position in the rectangle. Serial sections, 7 μ thick, were cut in paraffin transverse to the long axis of the wound, and these were stained with hematoxylin and eosin.

Considerable difficulty was encountered in identifying the healed wounds in the older specimens and this was never done successfully beyond the 6-day stage. Repair is so rapid and perfect that many preparations had to be discarded because of uncertainty as to the exact site and its marginal limits. Often, however, advantage could be taken of some anatomical clues. Once the linear extent of the wound had been established in serial sections, a location was chosen about midway in the series and twenty-five consecutive sections were then studied on each side of this level. Each transverse section was scanned carefully with the oil immersion objective throughout its extent, and all examples of mitosis at any stage (including early prophases) were recorded. These enumerations, therefore, represent a census of the epithelial area 1 to 2 mm. on either side of the wound, as well as the newly epithelized

² Carleton ('34), studying the rate of mitosis in the epidermis of recently born white mice, was able to detect a daily rhythm with a maximum at 8 P.M. to midnight and a minimum at about noon. The average ratio was 2.7: 1. Picón ('33), on the contrary, found the maximum number of mitoses in the mature mouse at noon and only one-third this number between 8 P.M. and midnight. In the present experiment the rats were killed during the daytime.

region itself. To compare fairly the several mitotic frequencies, the amount of epithelium represented by each set of fifty sections was computed and the number of mitoses was then translated into standard indices of reference. These were established by using either 1 cu. mm. or 1 sq. mm. of epithelium as a constant unit of measure.

For controls, two counts were made on the normal corneas of two adult rats, while to test a possible age difference another count was made on a sexually immature animal. By way of comparison, further counts were made on the stratified epithelium of the esophagus of the same adult rats.

Much corneal material was prepared that could not be used for the specific purpose of making mitotic counts. Thus, ten series between 7 and 20 days were unusable because the wound site could not be identified through complete and perfect healing. In addition, thirteen series between 54 and 171 hours were eliminated because of minor unfitnesses, such as too deep a lesion or too poor centering of the wound site. In the end, nine entirely suitable stages were selected for counting. These were taken at the following times after infliction of the standard injury: 6, 12, 24, 48, 75, 96, 120, 192 and 240 hours.

OBSERVATIONS AND DISCUSSION

Uninfected corneal wounds heal remarkably fast. In the 6-hour stage the floor of the denuded area was half covered with epithelium. At 12 hours (and all later stages) the defect was wholly covered. The actual area involved (1.5 sq. mm.) was, of course, not large, yet it constituted some 7% of the total area of the cornea. In the only infected specimen, the 59-hour wound was still unclosed. Another cause of healing delay, which led to rejection from use, is injury of the fibrous basis of the cornea resulting in perforation and loss of fluid from the anterior chamber; this destroys the natural substrate, produces faulty approximation, and results in a "jammed" mound of epithelial cells at the wound margins.

In the majority of instances the advancing epithelial sheet tapers to a marginal wedge. The pointed edge is composed of

flat cells with darkly stained nuclei. Except at the tip, the elements of this region are not arranged in the customary orderly manner, and distinctive basal cells are not seen. A little farther peripherad, where basal cells are recognizable as such, they stand obliquely rather than in a normal, vertical direction. Loeb (1898) described in the healing of skin wounds how the darkly staining nuclei from the granular and horny layers occur in a syncytial mass that moves first and fastest onto the wound site, whereas the more slowly migrating Malpighian cells become elongate spindles. Although our material shows many of the advancing epithelial margins to be wedges of more or less flattened cells, it does not prove conclusively that these cells do not derive from both the originally flatter cells and the more basal cells as well.

In some series the epithelial margin is club-shaped and the cells are massed without apparent order. In these instances, however, exudate occurs over the base of the wound, and it would seem, as though in an attempt to surmount this obstacle, the cells have piled up in the manner described. It is much the same picture as is seen when infection causes hindrance to the rapid movement of cells across the denuded surface. In both of these conditions the impression is gained that a kind of mass movement is taking place and that the impelling force is sufficient to cause a "pile up" of cells when the progress of vanguard cells is hindered or arrested.

The epithelial surface, with the open wound in its center, can be roughly divided into three concentric zones: 1. An innermost, marginal strip; it may be thinned to a wedge (with a tip of flattened, parallel cells) or thickened at its rim (with indiscriminately massed cells). 2. Next peripherad is a noticeably thinned-out area, averaging two to three cells in thickness; the deeply-located, unflattened cells are usually reduced to a single row of block-like cells which are somewhat small in size. 3. Farthest peripherad is normal epithelium; the columnar basal layer is overlaid with one or two strata of polyhedral cells, and these, in turn, are topped by several layers of flattened cells. No accurate record was kept of the

numbers of mitotic figures encountered in these three zones, but, in the earlier stages, it was clear that mitoses were lacking in the first zone, rare in the second and usually were found in the third zone at the periphery of the section. Older stages show less sharp differences in the three zones, but so long as the wounded area can be identified easily, the general correlation still holds as stated. This is in partial agreement with the observation of Gurwitsch ('26), on the frog's cornea, that although mitoses appear in no trifling number in the vicinity of a wound, the maximum intensity of cell division occurs at a quite considerable distance from the wound wall.

The general observations recorded thus far imply that the prompt closure of these small wounds must be accomplished by cell migration, in which the process of cell division at the

TABLE 1
Frequency of mitosis in the normal cornea of the white rat.

	MITOSES COUNTED IN 50 SECTIONS	VOLUME IN CU. MM. OF EPITHELIUM EXAMINED	CALCULATED MITOSES IN 1 CU. MM. OF EPITHELIUM	AREA IN SQ. MM. OF EPITHELIUM EXAMINED	CALCULATED MITOSES IN 1 SQ. MM. OF EPITHELIUM
Adult rat no. 1	1509	0.0885	17,050	1.860	811
Adult rat no. 2	751	0.0454	16,542	1.089	689
Immature rat	712	0.0447	15,928	1.015	701

immediate site of the wound plays no important rôle. This implication makes it desirable to examine more closely the mitotic rate both normally and during the healing course. The frequency of mitoses in the normal cornea of the white rat is indicated in table 1. Two computations were made: one was on the basis of the number of mitoses in 1 cu. mm. of corneal epithelium; the other was on the basis of the number of mitoses in 1 sq. mm. of surface area. It will be seen that the averages for two large, mature rats were about 16,750 per cubic millimeter and 750 per square millimeter. The number of dividing cells in a cubic millimeter of tissue seems very large until it is remembered that the epithelium is only 40 μ thick and accordingly this total represents the mitotic content in about 25 sq. mm. of corneal area. A single determination on

one sexually immature animal (less than 40 days old) showed no marked difference in mitotic frequency. Actually the result was slightly under that of the older animals, rather than considerably higher as Boweri ('29) surmised might be the case. For the epidermis of the mouse (Picón, '33) and of the child (Thuringer, '28) it has been claimed that a decline in mitotic frequency occurs between birth and puberty. It is, therefore, entirely possible that our one test is unrepresentative, but there is still another interpretation (p. 82) that cannot be eliminated from consideration.

One is impressed with the relative abundance of mitotic figures encountered in a normal cornea. To obtain a better

TABLE 2
Frequency of mitosis in the normal esophagus of the white rat.

	MITOSES COUNTED IN 50 SECTIONS	VOLUME IN CU. MM. OF EPITHELIUM EXAMINED ³	CALCULATED MITOSES IN 1 CU. MM. OF EPITHELIUM ³
Adult rat no. 1	182	0.0406	4,482
Adult rat no. 2	162	0.0367	4,414

³ Most of the cornified layer was omitted, to make a fairer comparison with the corneal epithelium.

comparison with a stratified epithelium of another sort, counts were also made on the esophagus of the rat. It might be objected, with reason, that the epithelium of the cornea and esophagus cannot be compared fairly on the basis of volume, since the latter contains many more layers of flattened cells in which mitoses do not occur. Hence the esophagus would be expected to exhibit mitosis less frequently in relation to unit volume. In calculating the volume of the esophageal epithelium, however, most of the flattened strata were omitted. The deeper portion, which was measured, was reasonably comparable to the corneal epithelium in layering. The data of table 2, when compared with table 1, show that cell division was four times more abundant per unit volume in the normal cornea than in the esophagus. This evidence substantiates the

impression of unusual proliferation that is gained from a general visual inspection of the corneal sections, and it is in harmony with Boveri's ('29) somewhat ambiguous comment on the exuberant mitotic activity of this tissue. Moreover, the average of 734 mitoses per square millimeter in the adult corneal epithelium of the rat should be contrasted with the values ranging from 50 to 275 per square millimeter found by Picón ('33) in the epidermis of the mature mouse. It is possible that the greater abundance of mitoses in the cornea is a mere retention of the generally high mitotic frequency characteristic of tissues in general in the fetus and the young. In other words, the cornea may not suffer a proliferative decline comparable to that demonstrated by most somatic tissues.

TABLE 3

Frequency of mitosis after corneal wounding in the white rat.

INTERVAL IN HOURS	MITOSES COUNTED IN 50 SECTIONS	VOLUME IN CU. MM. OF EPITHELIUM EXAMINED	CALCULATED MITOSES IN 1 CU. MM. OF EPITHELIUM ⁴	AREA IN SQ. MM. OF EPITHELIUM EXAMINED	CALCULATED MITOSES IN 1 SQ. MM. OF EPITHELIUM ⁵
6	196	0.0275	7,127	1.085	181
12	194	0.0313	6,198	1.169	166
24	242	0.0271	8,934	0.935	251
48	338	0.0348	9,710	1.047	332
75	332	0.0343	9,869	1.637	249
96	517	0.0361	14,320	0.975	532
120	1,322	0.0511	25,870	1.920	689
192	872	0.0526	16,577	1.679	519
240	595	0.0476	12,500	1.344	443

⁴ The normal number is about 16,750.

⁵ The normal number is about 750.

Table 3 consolidates the quantitative data on mitotic activity both during epithelization (less than 12 hours) and during the 9½ days that followed. The mitotic course is also presented graphically in figures 1 and 2. Notable are the sharp decline in mitosis that follows straightway upon the infliction of a wound, the retention of this depressed rate for 3 days (long after re-epithelization is finished), and the upsurge on the fourth day. The readiest interpretation of the relatively long period of mitotic depression is in terms of shock and the

sequellae of trauma. Gurwitsch ('24) has also recorded the wide-spread occurrence of mitosis in the frog's cornea 4 days after inflicting small wounds by cautery.

Figure 1 shows that the mitoses at 5 days exceed considerably the normal number when unit volume is taken as the standard of reference. This result, however, is correlated directly with the thinning of the epithelium that is a concomitant of cell loss through emigration. Since the dividing cells are usually found in the basal layer only,⁶ it would thus appear that enumerations on the basis of area offer a more significant means of comparison than do those based on volume. Referring also to figure 2 it is found that the two curves are quite similar regardless of whether the standard of reference is area or volume, but that the relation to the normal rate differs somewhat. The initial drop below normal is relatively greater (to 22% as compared with 37%) in the graph plotted with respect to area, and the recovery is somewhat short of normal rather than above normal.

That cell division is not an important factor in the immediate healing of small corneal wounds is apparent. It can be calculated that the cell loss in the present experiments totalled about 100,000 to each cornea. Not only is the time needed for repair inadequate to produce this number of cells locally, but also the mitotic rate is lowest during the period of epithelial restoration. That the alternative method of repair is one of cell migration is attested by collateral evidence such as the thinning of the bordering epithelium. This general picture is in full accord with facts already established by one of us (Arey, '32) on the healing of small wounds in fishes and amphibia.⁷ It also coincides with the conclusion of Salzer ('11) and Gurwitsch ('24), as opposed to Jusélius ('10) and Törö ('32), that cell migration is primary in corneal repair and cell division is secondary.

⁶ This is true of the normal epithelium. In the thinner epithelium after migratory healing the polyhedral cells above the basal layer are less abundant and mitoses here are still less frequent.

⁷ In table 3 of the 1932 publication, which shows the time and frequency of this late revival of mitosis, there is a typographical error. Days should replace hours in designating the time stages.

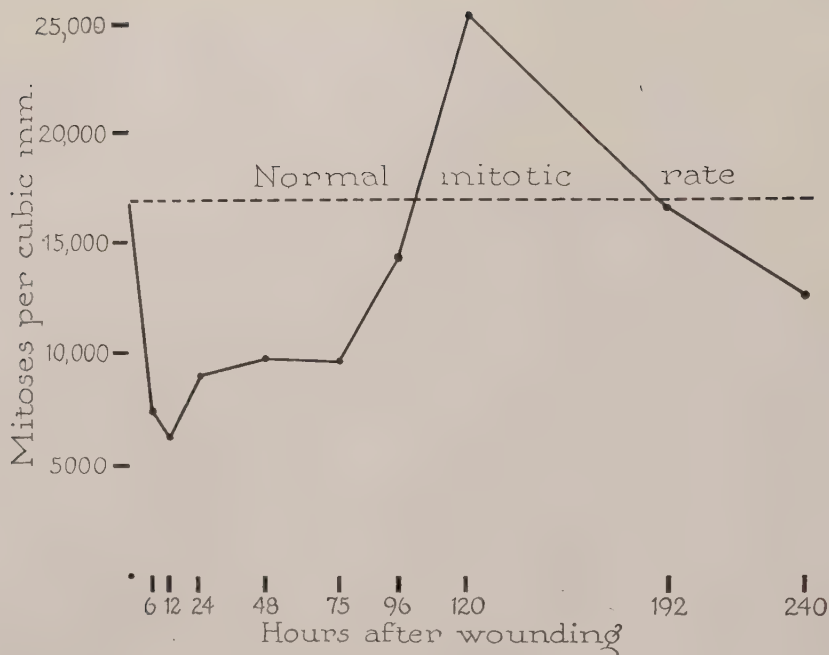


Fig. 1 The frequency of mitosis after corneal wounding, based on the number of mitoses per cubic millimeter of epithelium.

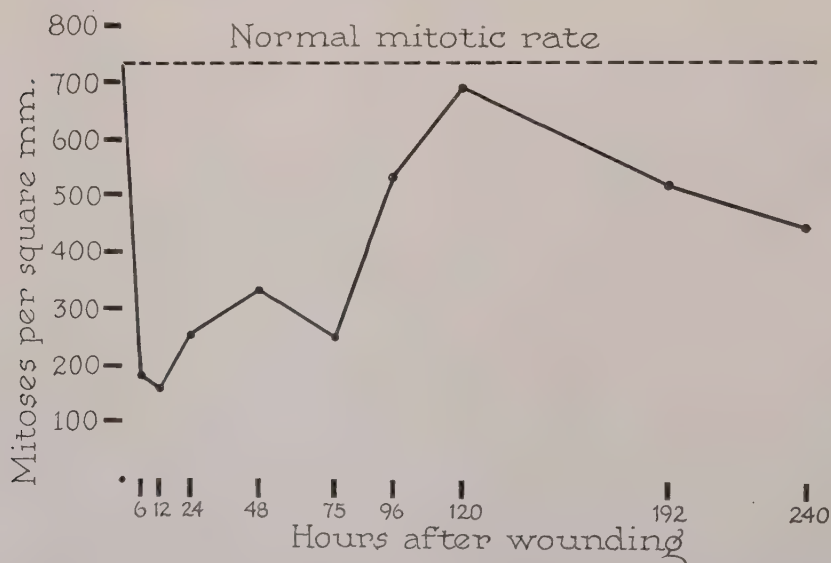


Fig. 2 The frequency of mitosis after corneal wounding, based on the number of mitoses per square millimeter of epithelium.

CONCLUSIONS

The repair of wounds in the corneal epithelium of the white rat is accomplished by cell migration which fills the defect promptly. During this primary period of re-epithelization the mitotic rate falls far below normal in the neighboring epithelial territory, while cell division is lacking, or virtually so, in the area actually being reclothed.

Although the new epithelial cover was found to be complete within 12 hours, it was not until 96 hours that the reduced mitotic rate increased again markedly. Even then it was in the more remote epithelium, rather than in the wound site proper, that mitoses occurred in any abundance. The greatest mitotic activity was shown at 120 hours.

During the 6 days under observation the frequency of cell division, as calculated on the basis of unit epithelial area, never exceeded the normal rate. Epithelial area, rather than volume, is the more significant basis for comparison.

The occurrence of mitosis in the normal cornea of the white rat is about 750 per square millimeter of epithelium, and about 16,750 per cubic millimeter. A young, sexually immature rat showed no significant difference in mitotic frequency from two older animals. Cell division is relatively frequent in corneal epithelium — about four times the rate in the esophagus. It is possible that the cornea merely retains the vigorous proliferative capacity which is enjoyed by young tissues in general, but which is greatly reduced at maturity in most of these tissues.

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CHANGES IN THE ACINAR CELLS OF THE PANCREAS IN RESPONSE TO THE PRESENCE OF PEPTONE IN THE SMALL INTESTINE

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TWO PLATES (SIX FIGURES)

INTRODUCTION

The mechanism through which products of protein digestion stimulate the external secretory function of the pancreas has not been determined. Thomas and Crider ('41) have recently shown that the peptones cause a flow of concentrated pancreatic juice within a few minutes after being placed in the intestine. Because the specific gravity and nitrogen content of the secretion resulting from peptone stimulation are greater than can be obtained with secretin and are comparable to those of the juice resulting from stimulation of the vagus nerves, it was assumed that a nervous mechanism is involved in the reaction to peptone. Partial confirmation of this assumption was obtained in experiments in which it was found that the usual response of the pancreas to peptone stimulation was not obtained immediately after double cervical vagotomy or cocainization of both vagi in the neck (Crider and Thomas, '42). The experiments were not continued for a sufficiently long period of time after vagotomy to warrant a definite conclusion.

The present study was undertaken to obtain additional information through the study of histological changes in the pancreas during secretion caused by the action of peptones in

the intestine. Babkin, Rubaschkin and Ssawitsch ('09) and, more recently, Sergeyeva ('38) have demonstrated characteristic changes in the acinar cells of the pancreas following prolonged stimulation of the vagus nerves. On the other hand, according to the former authors, the changes following hormonal stimulation (HCl in the intestine) were of a different character and less pronounced. We undertook to determine whether the presence of peptones in the intestine caused changes resembling those known to follow stimulation of the vagus nerves, and if so, whether the results were modified by vagotomy.

METHODS

Preparation of the animals. The experiments were performed on unanesthetized dogs in which a portion of the pancreas had been exteriorized and an injection tube inserted into the duodenum. The necessary surgical operations were performed under ether anesthesia 2 days before beginning the observations.

To exteriorize the pancreas a semicircular incision was made through the skin and superficial fascia of the lateral abdominal wall so placed that the mid-point of the arc was about 2 inches lateral to the umbilicus with its two ends pointing dorsad. The included skin and subcutaneous tissue were elevated, exposing the underlying muscles. The muscles and peritoneum were incised along the base of the denuded area and the duodenum and part of the pancreas brought out through the incision. A portion of the pancreas where it parallels the descending duodenum was mobilized by separating it from the duodenum and its peritoneal attachments. It was then sutured to the subcutaneous tissue along the concavity of the semicircular skin incision so that part of it lay on the external surface of the exposed muscles. As a rule no blood vessels supplying the pancreas were severed. The sutures did not pass through the pancreatic tissue but were confined to the marginal peritoneum.

To provide a means of injecting peptone solutions into the intestine a piece of "spaghetti" tubing was tapered at one

end and threaded into the eye of a large needle which was then used to draw the tapered end of the tube into the duodenum. The needle was recovered and used to draw the other end of the tube out through the abdominal wall in the same manner. In a few experiments the injection tube was inserted through a small duodenal fistula.

Finally, after replacing the duodenum, the incision through the abdominal muscles was closed as far as possible without constricting the emerging pancreas, and the skin flap was sutured in place. The dogs were given water but no food for 72 hours preceding the observations.

Eleven dogs were operated upon in this manner, of which four (dogs 1, 2, 3 and 4) were used without further surgical preparation. In five of the eleven the vagus nerves were severed. In four of these (dogs 6, 7, 8 and 9) the right vagus was cut below the origin of the recurrent nerve and the left vagus was exposed in the neck and a strip of Cellophane, looped around it, was allowed to protrude from the wound. On the following day the left vagus was brought into view by gentle traction on the Cellophane, and severed. In one of the five (dog 5) both vagi were prepared as was the left vagus in the other four and were cut in the neck on the morning of the experiment. This animal became violently ill and vomited frequently.

In the two remaining animals the duodenum was separated from the stomach in order to determine to what extent the results observed in the other animals were influenced by the passage of gastric juice into the intestine. In one (dog 10) the separation was made at the pylorus and fistulas made of the pyloric ends of the stomach and duodenum. This animal did not react well and was obviously ill on the day of the experiment. In the other (dog 12) the pyloric portion of the stomach was divided about $1\frac{1}{2}$ cm. from the pylorus and a fistula made of the lower end of the upper segment of the stomach. The upper end of the lower segment was closed with sutures and left in situ. This animal remained in excellent condition. In both of these animals the vagi were left intact.

Experimental procedure. On the day of the experiment, after injecting procaine into the skin surrounding the wound, the cutaneous sutures were removed and the skin flap lifted up, exposing the exteriorized pancreas, a small sample of which was excised for histological study. Twenty cubic centimeters of a 5% peptone ("Bacto Protone") solution were then injected into the duodenum every 10 minutes for from 4 to 6 hours. Additional samples of pancreatic tissue were taken for study from time to time, generally at the end of each hour. In six of the experiments (dogs 1, 3, 4, 9, 10 and 12) no peptone was injected for a period of approximately 1 hour between the second and fourth hours. In these an opportunity was provided for observing whether a noticeable degree of recovery occurred in the pancreas during a 1-hour rest period.

Histological methods. Before experiments on prepared animals were begun, several tests were made to determine the most reliable fixation and staining methods for demonstrating zymogen granules in the pancreas of the dog. Thin pieces of pancreas taken from an anesthetized animal were fixed in Zenker-formol solution (Bensley) and in acetic-osmic-bichromate solution (Bensley). The tissue was embedded in paraffin (Bioloid, 56°–58°C.) and cut at 3 μ and 5 μ . It was found that, following spreading, sections left overnight in a slide drying oven (at 50°C.) adhered to the slides much better than those not so treated. Pancreas samples fixed by the Zenker-formol solution were stained with hematoxylin and azure-eosin, neutral gentian, Bowie's stain and crystal violet-acid fuchsin. Sections fixed in the acetic-osmic-bichromate fluid were stained with safranin-acid violet. (The procedures for these staining techniques were taken, essentially, from the Bensleys' Handbook of Histological and Cytological Technique.)

Neutral gentian gave the most consistent results and was accordingly chosen for routine staining although two others, Bowie's stain and crystal violet-acid fuchsin, gave good results and were employed in some cases, also. Samples of pancreas large enough to present a representative picture of

the functional condition of the gland were found, at times, to be too large for perfect penetration by the fixing fluids. To obviate this difficulty, the fixing solutions were quickly injected into the larger samples (using a 27-gauge needle). Following injection, the tissues were sliced into thin pieces with a sharp razor blade, then placed in fixing fluid in the usual manner. At the termination of each experiment the animal was anesthetized and samples were taken from that part of the pancreas which remained inside the abdomen so that comparison with those from the exteriorized portion of the gland could be made. Intra-arterial injection of fixing fluid was utilized, occasionally, for the taking of the final samples.

RESULTS

1. *Animals with intact vagus nerves and normal gastro-duodenal continuity (dogs 1, 2, 3 and 4).* Samples of pancreas procured at the beginning of the experiments, before injection of the peptone solution, showed the usual fasting condition. The acini were well charged with zymogen granules (fig. 1) which were distributed from the apices of the cells to the basal (outer, chromidial) zone. The granules were remarkably uniform in size. Seldom could one find any secretion in the intralobular ducts although the presence, in the smaller ducts, of minute amounts of secretion has been reported often and considered normal for the fasting state in the dog.

Samples taken at the end of the first hour, after 120 cc. of peptone solution had been injected into the duodenum, showed definite secretory activity. This was evidenced by the grouping of zymogen granules toward the apices of the cells (near the central lumina of the acini) and, also, by the presence of secretion in the small ducts near the acini (fig. 2). Samples taken at hourly intervals from the second hour to the end of the experiment showed advanced secretory stimulation. Marked diminution in the number of granules, their location at the apical ends of the cells, and increased amounts of stainable secretion in the ducts were noted in all cases (figs. 3 and 4). Acinar cells in the vicinity of islets were less

affected by peptone stimulation and in two of the four animals stood out as deeply stained areas in sharp contrast to the surrounding tissue (figs. 4 and 5). These areas correspond to the halos described by Sergeyeva ('38) which become evident following stimulation of the vagus nerves.

The histological appearance of the pancreas following 3 to 6 hours of peptone administration resembled very much the results obtained by vagal stimulation.

It is difficult to interpret the samples taken following an interruption, for 1 hour (usually during the third hour of the experiment), in the peptone injection. In one instance a definite increase in the number of zymogen granules was noted. Whether the increase was due to actual recovery or to a marked functional variation in that particular portion of the gland from which the sample was taken, could not be determined. It is assumed that some peptone solution would remain in the intestine for a time and would, therefore, furnish continued pancreatic stimulation, although no additional solution was administered.

Samples from the exteriorized part of the gland were strikingly comparable to those taken from the region which remained inside the abdomen. The blood supply of the exteriorized portion seemed unimpaired since a free oozing of blood always occurred when a sample was obtained from the subcapsular area and, further, typical arterial bleeding was encountered whenever a deep sample was taken.

2. *Animals with severed vagus nerves (dogs 5, 6, 7, 8 and 9).* The samples of pancreas from dogs in which the vagus nerves were severed before the administration of peptone solution, showed no striking histological changes as a result of injecting peptone into the intestine. Preliminary samples were usually quite typical of the fasting state of the gland although some areas suggested slight secretory activity. Specimens obtained at hourly intervals throughout the experiments exhibited no apparent loss of granules nor was any other definite evidence of secretion noted (fig. 6). To the contrary, a recognizable shift toward a more characteristic fasting appearance was

manifest in the later and last samples from those animals (three of the five dogs in this group) in which the initial state of the gland was not that of extreme fasting.

Samples from the two regions of the pancreas (exteriorized and abdominal) were found in this group, also, to present similar and comparable histological pictures.

3. *Animals with intact vagus nerves but with stomach and duodenum separated (dogs 10 and 12).* One of the two dogs in this group (dog 10) was ill at the beginning of the experiment. Accordingly, the experiment was terminated too soon to be of value. Only two hourly samples were taken; both showed typical fasting conditions. The pancreas of the other animal (dog 12) manifested definite secretory stimulation. Thus, stimulation of pancreatic secretion from the presence of peptones in the duodenum, without any possible additional influence through the gastric contents reaching the duodenum, is clearly demonstrated by this experiment. However, the degree of depletion of zymogen granules in this animal seemed to be a little less than the average attained in experiments where stomach and duodenum were in normal communication.

DISCUSSION

Our interpretation of these results is based on the assumption that the so-called zymogen granules in the acinar cells of the pancreas comprise much of the materials which ultimately appear in the secretion as enzymes or proenzymes. The assumption is probably justified even in the absence of direct positive evidence to support it. The similarity of the reactions of the zymogen material and the secretion in the ducts to fixatives and stains suggests identity or close similarity in their chemical composition. It has been shown that stimuli which bring about the formation of secretion rich in enzymes (vagus stimulation or the presence of peptone in the intestine), also cause depletion of the granules, whereas stimuli which bring about a more dilute secretion (secretin and HCl) leave the granules largely intact (Babkin, Rubaschkin and Ssawitsch, '09). These facts further suggest that depletion

of the granular material in the acinar cells is brought about by its actual transfer to the secretion in the ducts.

The results obtained in the animals with vagi intact (dogs 1, 2, 3 and 4) show that the presence of peptone in the intestine has an effect on the acinar cells of the pancreas similar to that brought about by electrical stimulation of the vagus nerves. They are, therefore, in accord with the hypothesis that peptone stimulates the pancreas through a nervous mechanism.

The significance of these experiments is not diminished by the fact that in some of the animals the secretagogue action of peptone may have been augmented by gastric HCl. Changes in the pancreas of the type observed in these experiments are not brought about by the action of HCl alone (Babkin, Rubaschkin and Ssawitsch, '09). Furthermore, good evidence of secretory stimulation was obtained in the one satisfactory experiment in which gastric juice was excluded from the intestine. The fact that in this animal the changes in the cells were less pronounced than in a majority of the others accords with the reasonable assumption that a combination of HCl and peptone is a more effective stimulus than peptone alone.

The failure of peptone stimulation to affect the acinar cells in vagotomized animals (dogs 6, 7, 8 and 9) indicates that in the normal animal the mechanism responsible for the observed changes in the acinar cells is in some manner dependent on the integrity of the vagus nerves. A more specific conclusion on this point is not warranted at the present time because two of us (Crider and Thomas, unpublished) have found that the secretory response of the pancreas to peptone partially recovers, after a time, in vagotomized animals.

SUMMARY AND CONCLUSIONS

1. Using unanesthetized dogs with partially exteriorized pancreas, it has been shown that the presence of peptone in the intestine causes characteristic changes in the acinar cells of the pancreas.

2. During peptone stimulation the acinar cells become depleted of zymogen granules and secretion accumulates in the ducts. Acinar cells in the vicinity of islets retain their granules, some forming halos.

3. The changes observed are similar to those which have been described as occurring during stimulation of the vagus nerves and differ from those which are said to result from the presence of HCl in the intestine (hormonal stimulation).

4. Characteristic changes did not occur during peptone stimulation in vagotomized animals.

5. The results of these experiments are in accord with the hypothesis that peptone stimulates the pancreas through a nervous mechanism which is dependent, for its normal irritability, on the integrity of the vagus nerves.

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PLATE 1

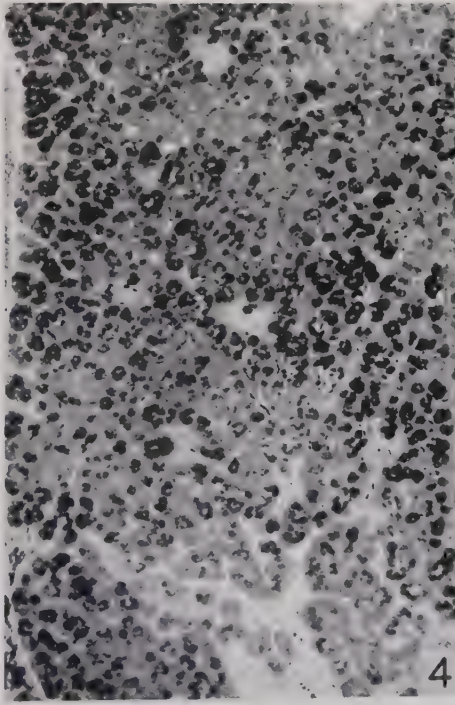
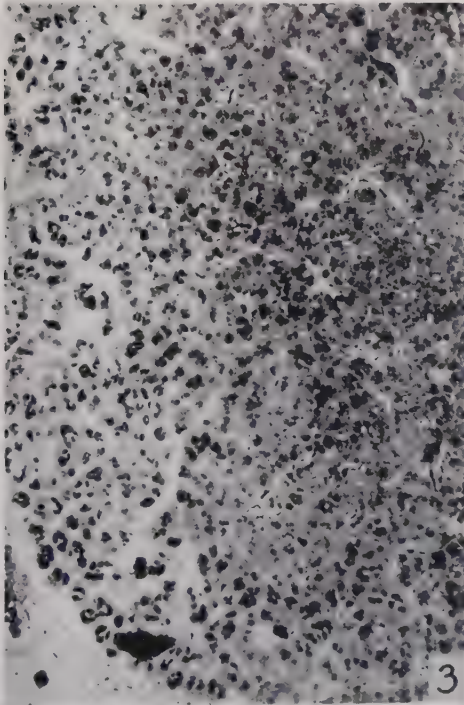
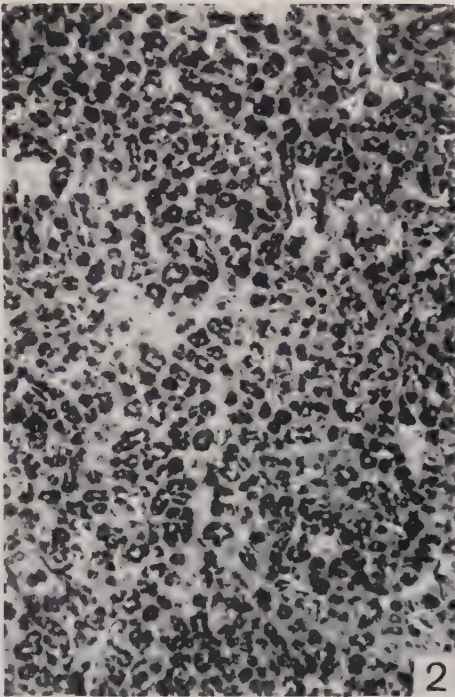
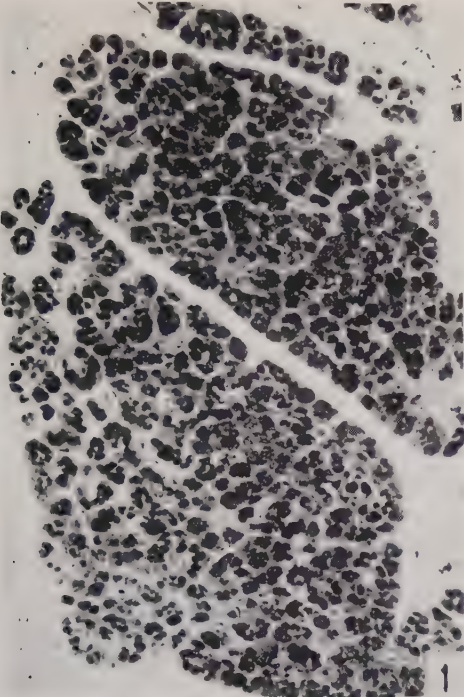
EXPLANATION OF FIGURES

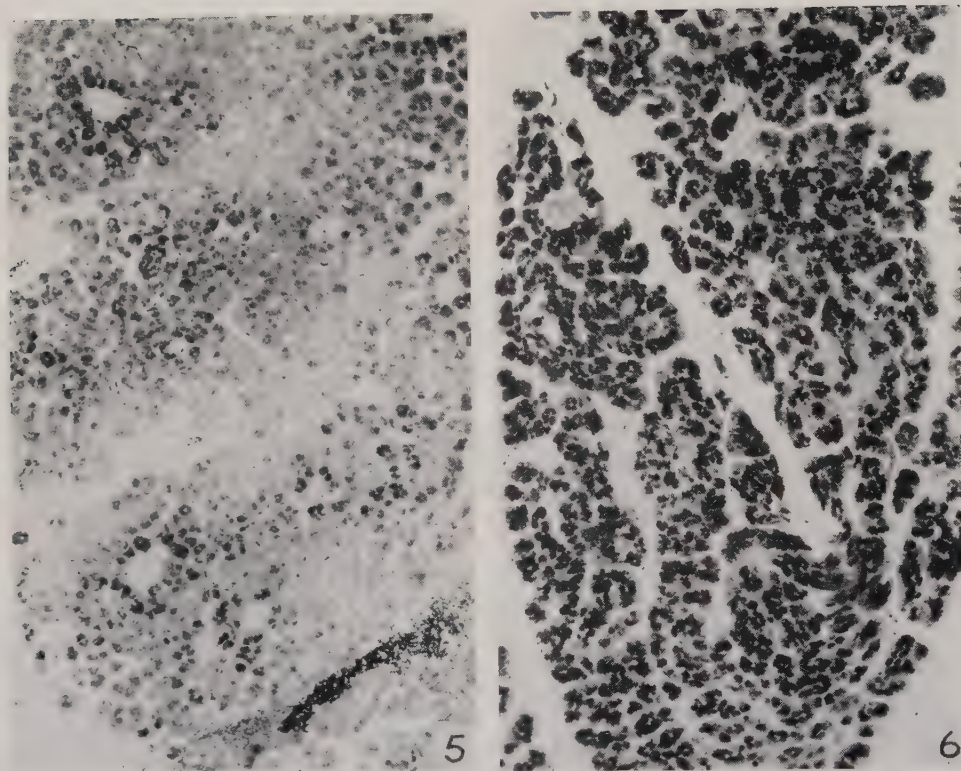
1 Two peripheral lobules of the pancreas of a dog which had fasted for 48 hours. Note that the acini are large and well charged with zymogen granules. Two ducts, in the lower lobule, contain some stainable secretion. The vagus nerves were intact. $\times 95$.

2 A large pancreatic lobule after administration of peptone solution for 1 hour. The clumping of granules centrally in the acini and the appearance of considerable amounts of stainable secretion in the ducts illustrate secretion stimulation. The vagus nerves were intact. $\times 95$.

3 Depletion of granules and large amounts of secretion in the ducts of a dog's pancreas following 2 hours of peptone administration. No islets of Langerhans are seen in this field. The vagus nerves were intact. $\times 95$.

4 Pancreas after 3 hours of the peptone treatment. Some acini have lost nearly all of their granules, others apparently have lost but few. Several islets may be seen with suggestions of halos of well charged acini surrounding them. The vagi were intact. $\times 95$.





5 Marked depletion of zymogen granules after administration of peptone solution for 5 hours. All the small ducts contain secretion. The sample was taken from the peripheral (subcapsular) part of the gland, therefore no large ducts are shown. The halos of unaltered acini around the islets are seen clearly. The vagi were intact. Cut at 3 micra. $\times 95$.

6 Pancreas of a dog in which the vagus nerves were cut prior to the experiment. This sample of pancreas, taken after 5 hours of peptone solution injection, exhibits no signs of secretion stimulation. Compare with figure 5. Two small islets are visible in the field. Cut at 6 micra. $\times 95$.

THE DETERMINATION OF THE CONCENTRATION OF SPERMATOOA IN FOWL AND BULL SEMEN ¹

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TWO FIGURES

The development of laboratory methods for the determination of fertility in the male and for the measurement of semen quality have been markedly improved during the past decade. Although no single measurement of semen quality such as volume of semen, motility of spermatozoa, abnormal sperm forms, the ability of spermatozoa to survive in unfavorable environments, pH, or the concentration of spermatozoa per unit volume of semen, can be used for the accurate estimation of relative fertility, the application of all of these methods yields valuable information on semen quality.

The determination of the number of spermatozoa in semen is usually performed with the hemocytometer by the methods described for counting red blood cells. This method is subject to considerable personal error even when employed by experts, and gives extremely variable results when used by the average technician. Berkson, Magath and Hurn ('40) in a critical study of the error of estimate of the blood cell count as made with the hemocytometer found that, even when the actual counting error was eliminated by taking photomicrographs of the cells, the coefficient of variation of the remaining error was still 7.8%. Belding ('34) as quoted by Weisman ('41) reported that in 600 counts of fifteen semen samples at least

¹ Journal Paper No. 64, Purdue University Agricultural Experiment Station.

half varied from the mean $\pm 6\%$, that one of each three counts varied $\pm 10\%$ and that one out of nine varied about $\pm 20\%$.

Since the volume of red blood cells can be determined by the centrifugation of non-coagulated blood in hematocrit tubes it was decided to investigate the use of this technique in the determination of sperm cell volume in semen.

PROCEDURE

Over 1000 tubes of various diameters and lengths have been used in the determination of the volume of spermatozoa in samples of fowl and bull semen. Since the amount of fowl semen per ejaculate is seldom more than 0.8 cc., and since the necessity of saving the greater portion of the semen for physiological studies makes it impossible to use the entire ejaculate for the determination of cell volume the problem was one of selecting capillary tubes of the minimum capacity compatible with the greatest accuracy.

Capillary tubes approximately 7 cm. long with an average inside diameter of 0.5 mm. and of the type used for blood coagulation time determinations were found to be most satisfactory for this work. The tubes were sealed at one end in a flame and a 3- to 4-mm. column of mercury was placed at the sealed end of each to serve as a base line for the semen column (fig. 1). The mercury was inserted with a capillary pipette and the tubes centrifuged sufficiently to throw the mercury to the bottom of the tubes.

The fowl semen samples were collected by the massage method as described by Burrows and Quinn ('37) and the bull samples by the use of the artificial vagina. The semen was shaken in a mechanical shaker for approximately 2 minutes, a portion immediately withdrawn and the capillary tubes filled with semen. This was accomplished either with glass capillary pipettes or with a 1-cc. syringe fitted with a 3-inch length of 26-gauge stainless steel hypodermic tubing (fig. 2). The steel tubing has proved to be more suitable because of its uniformity and durability.

Groups of five filled capillary tubes were placed in 75 mm. by 12 mm. serum tubes for purposes of group identification and centrifuged at 3000 R.P.M. for 15 minutes in a centrifuge

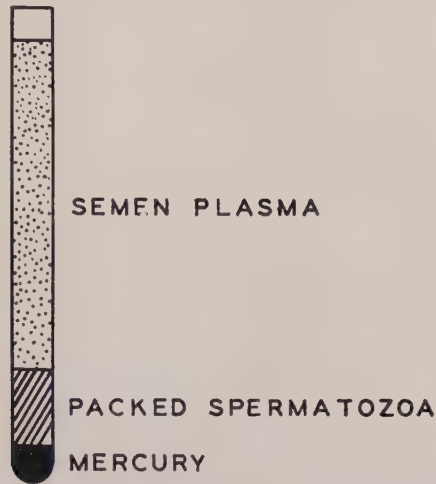


Fig.1 Diagrammatic appearance of semen in a capillary tube following centrifugation.

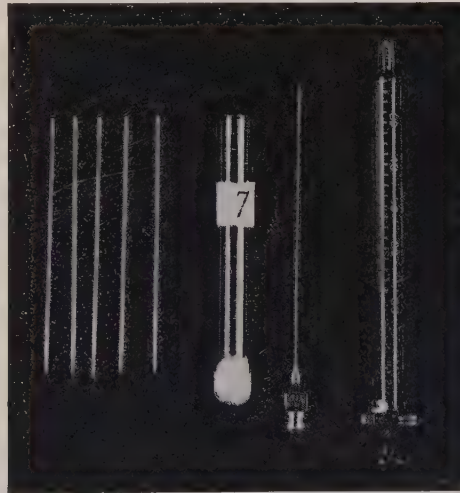


Fig.2 Photograph of equipment used in determination of sperm cell volume. Syringe, 26-gauge hypodermic tubing, numbered vial for identification of groups of tubes during centrifugation, filled capillary tubes.

with a 10-inch radius. After centrifugation the tubes were immediately placed on the mechanical stage of a microscope, the total length of the semen column and of the packed spermatozoa measured, and the per cent of the total column that was composed of cells calculated.

RESULTS

Effect of variation in the size of capillary tubes

Since it is very difficult to obtain capillary tubes of exactly 0.5 mm. diameter it was necessary to determine whether or not differences in sperm cell volume within the same semen sample were due to variation in capillary tube size. This was accomplished by identifying a group of tubes, obtaining the volume of spermatozoa of a particular semen sample, then thoroughly cleaning and drying the tubes and again obtaining the cell volume of the same semen in the same tubes. A comparison of the results of six trials showed that there was no consistent trend within individual tubes. A particular tube might give the greatest volume of cells in one trial and the lowest volume in a successive trial. In order that tube variation might be further tested sixty tubes were selected at random and used on a single semen sample. The percentage of sperm cells in each tube was determined and all tubes deviating more than 10% from the mean were eliminated. The remaining tubes were then cleaned and tested three successive times with semen from the same sample. Those tubes which deviated from the mean more than 10% were eliminated each time, yet the variation between tubes with the same semen continued. This indicates that the source of variation was not confined to the individual tubes used.

Tubes with inside diameters of 0.8 mm. and 1.0 mm. were compared with those of 0.5 mm., and the results were similar for the three sizes. The larger tubes were objectionable for fowl semen because of the limited volume of the ejaculate, but were well suited for bull semen. Approximately 0.10 cc. of semen is required to fill five capillary tubes of 0.5 mm. inside diameter.

Effect of storage time and temperature on sperm cell volume

It was observed that when more than a few hours elapsed between the time of semen collection and centrifugation the volume of cells appeared to increase. Six samples of fowl semen ranging in initial cell volume from 10.2 to 18.2% were stored in a refrigerator at 5–7°C. and examined on successive days. The subsequent centrifugation of these samples showed that at these storage temperatures a maximum volume of solid material was obtained about 30 hours after the collection of the semen, and that the volume of packed material increased approximately 30% during this time. When the fowl semen was immediately placed in the ice water bath and centrifuged at 4-hour intervals the increase in the per cent of solid material was negligible. When fowl semen was stored at approximately 25°C. and examined at 4-hour intervals it was found that a 25% increase in the volume of solid material occurred during the first 12 hours. Bull semen stored at temperatures of 5 to 7°C. and 25°C. and centrifuged at 4-hour intervals showed no consistent change during the first 56 hours. Although changes in O₂ and CO₂ tension and pH which occur during semen storage might result in actual changes in the size of spermatozoa, these changes might also result in the precipitation of some of the semen plasma protein thus affecting the observed volume of cells. The morphology of fowl spermatozoa is such that accurate measurements of the volume of the sperm cells is difficult if not impossible, and the causes of the increase in the volume of solid material are at present unexplained.

Variability of the method

The variability which occurs in laboratory procedures is of prime importance in the development of new techniques. The coefficient of variation (V) was used as a measure of relative variation and was calculated from the formula:

$$V = \frac{\hat{\sigma}}{m}, \text{ where } m \text{ is the grand mean}$$

$$\hat{\sigma} = \sqrt{\frac{\sum_{k=1}^N \sum (X - \bar{x})^2}{N-1}}$$

and $\hat{\sigma} =$

where N is the number of replicates for each sample and k is the number of samples.

The results which are presented in table 1 indicate that the variability is relatively small and that less variation was obtained with bull semen than with fowl semen.

TABLE 1
Coefficient of variability of sperm cell volume determination.

SPECIES	NO. OF TUBES CENTRIFUGED PER SAMPLE	NO. OF SEMEN SAMPLES	AVE. % OF CELLS	AVE. σ WITHIN SAMPLES	% COEF. OF V WITHIN SAMPLES
Fowl ²	5	21	14.26	1.33	9.33
Fowl ¹	5	20	16.51	1.30	7.87
Bull ²	6	7	8.53	0.55	6.45
Bull ¹	5	11	9.61	0.61	6.35
Bull ¹	5	46	11.22	0.61	5.44

¹ Tubes filled with 26-gauge hypodermic tubing.

² Tubes filled with hand drawn glass capillary pipette.

Correlation of sperm cell volume and hemocytometer concentration counts

One hemocytometer count was made on each of thirty-one samples of fowl semen by a skilled technician and the percentage of cells in the same samples of semen was determined by centrifugation. Five tubes of each sample were centrifuged and the average cell volume of each sample obtained. The mean number of spermatozoa per cubic millimeter of semen as determined with the hemocytometer was 3,260,000 $\pm$ 840,000 and the mean percentage of sperm cell volume of the same samples 13.7 $\pm$ 4.4. The coefficient of correlation of the two methods was 0.76 (significant at the 1% level).

The regression equation for predicting hemocytometer count from per cent of sperm cells by volume was found to be:

$$E = \bar{y} + 147,000 (X - \bar{x}) \text{ or} \\ E = 147,000X + 1,250,000$$

E = estimated number of sperm. Y = hemocytometer count. X = per cent of cells by volume.

The same comparisons were made on forty-five samples of bull semen by a second trained technician. The mean number of sperm per cubic millimeter of bull semen was $950,000 \pm 47,000$ and the mean percentage of sperm per unit volume of semen was 11.5 ± 5.5 . The coefficient of the correlation of the two methods was 0.80 (significant at the 1% level).

The regression equation was:

$$\begin{aligned} E &= \bar{y} + 85,000 (X - \bar{x}) \text{ or} \\ E &= 85,300X + 381,000 \end{aligned}$$

If, for example, the volume per cent of cells in a sample of semen was 14, the expected number of cells determined with the hemocytometer would be $14 (85,300) + 381,000 = 1,575,000$ per cubic millimeter of semen.

DISCUSSION

The usefulness of routine laboratory methods for the determination of semen quality are dependent upon such factors as variability of the results under standard conditions, required operator skill, and time necessary for the determination.

The determination of sperm cell volume by the centrifugation of semen in capillary tubes of relatively uniform diameter and the measurement of the length of the column of packed cells appeared to yield more uniform estimates than the usual concentration count with the hemocytometer. If it were technically possible to make accurate determinations of sperm cell concentrations with the hemocytometer and sperm cell volumes by centrifugation a theoretically perfect correlation between cell volume and cell numbers should exist. It is to be regretted that more data are not available for a more detailed analysis of the two methods. As previously pointed out, single hemocytometer counts were compared with the average cell volume of five centrifuged samples. The calculated correlation coefficients of 0.76 and 0.80 for fowl and bull semens respectively, although significant at the 1% level, must be interpreted with certain reservations. The error of predicting sperm cell numbers by the measurement of cell volume is

potentially great, and its chief value would be in assisting the reader to visualize volume in terms of relative numbers until the centrifugation method had become widely used.

When the variability of the determination of cell volume by centrifugation was compared to the variability found by Berkson, Magath and Hurn ('40) for the hemocytometer determination of red blood cell concentration it was found that the differences in variability were slight. The coefficient of variability of the centrifugation method was slightly less for bull semen and slightly greater for fowl semen than the variability of the hemocytometer method. However, the highly refined technique of Berkson et al. involving the photographing of the blood cells in the hemocytometer is not comparable to the routine use of the counting chamber. The comparisons which have been made in the present study are on the basis of a single hemocytometer count and a single cell volume determination. Since five to ten tubes per semen sample can be handled simultaneously, the error of determination can be reduced by the square root of the number of tubes used. If six tubes were used for each determination, the fiducial limits would be reduced about one-fourth in comparison with the use of a single tube.

Since the personal error involved in counting spermatozoa in the counting chamber is eliminated by centrifugation, since the variability of the method can be markedly decreased by the use of a series of tubes, and since the time required for the simultaneous determination of cell volume in five to ten tubes is markedly less than that necessary for the determination of a similar number of hemocytometer counts, it would seem that the method should be well adapted for routine laboratory use.

SUMMARY

1. There were no significant variations in sperm cell volume when determinations were made with capillary tubes with inside diameters of 0.5 mm., 0.8 mm., or 1.0 mm.

2. There was a 25% increase in the volume of solid material thrown down by centrifugation when fowl semen was stored for 12 hours at 25°C. This increase did not occur when fowl semen was stored in ice water.

3. Bull semen stored at 25°C. for 56 hours showed no significant changes in the volume of solid material obtained by centrifugation.

4. The average coefficient of variability of the determination of sperm cell volume was 7.87% for fowl semen and 5.40% for bull semen.

5. The coefficients of correlation of the hemocytometer concentration count and the centrifugation volume determination were 0.76 and 0.80 for the fowl and bull semens respectively.

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EXPERIMENTS CONCERNING THE MECHANISM OF PITUITARY COLLOID SECRETION

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ONE PLATE (FIVE FIGURES)

Although a definite intermediate or middle lobe is not distinguishable in the adult human pituitary the borderline region between the anterior and posterior lobe is occupied by numerous cysts whose irregular cavities are filled by colloid. This homogeneous material is similar to that seen in the pituitary cleft between the anterior and middle lobe of the adult rodent hypophysis. The cysts are mainly derived from the cleft in Rathke's pouch, a cleft which is usually present in infants and occupies most of the space inside the middle lobe. In most cases it is practically obliterated at puberty and in the adult the above mentioned small cysts take its place (Fraser, '21; Schönig, '26; Marburg, '29). While the cleft persists there is usually a distinct difference in the epithelium of its anterior and posterior wall. The anterior wall consists of three to four layers of cells, all of which show the characteristics of anterior lobe cells, some of them containing eosinophilic, others basophilic granules. The posterior wall, however, is usually lined with a simple layer of cuboidal or cylindrical ciliated cells. In cases in which a number of small cysts is present instead of a large one, the epithelium may be composed either of anterior lobe cells or of short cylindrical cells with or without cilia (Rasmussen, '29). If the cysts are very much distended with colloid, the lining becomes flat (Benda, '32). Guizzetti ('33) emphasizes the similarity between the lining cells of the cysts and the basophils of the

anterior lobe. Dayton ('26) described transitional cells between the respiratory epithelium of Rathke's pouch and chromophil cells in cyst walls. Rasmussen ('29) pointed out that besides ciliated cells, mucus-producing goblet cells are present in the lining epithelium of such cysts.

Contrary to the above mentioned investigators Stendell ('14), Guizzetti ('25, '27, '28), Kasche ('26) and Pietsch ('30) do not think that all the cysts in this region are derivatives of Rathke's cyst. In fact Pietsch ('30) goes so far as to deny any participation of the original cleft in the formation of these cystic structures, which, according to him, are hollow glandular buds arising from the superior margin of the original hypophyseal cavity. It is possible that both conceptions are partly right in as far as some of the cysts may come from the original cleft while others are derived from hollow glandular buds of the hypophyseal anlage. Cysts which are not lined by a regular epithelium should more likely be considered the result of cellular degeneration (Collin, '22). They probably belong to the anterior lobe and have no direct relationship with the above mentioned cysts.

The cysts of the middle lobe contain a considerable amount of yellow colloid material, the significance of which is not as yet well understood. Rogowitsch (1889) believed it to be a secretion of the granulated cells but Stieda (1890) denied this. There are small colored globules in the blood vessels of this region and in the tissue of the posterior lobe and stalk. These have been considered to be identical with the colloid inside the cysts and the fact that these bodies are found all the way up to the tuber cinereum led to the theory that the hypophysis secretes directly into the nervous system (Collin, '33; Cushing, '33).

Histologically the various colloid accumulations may be largely different. Variations in their optic refractions and dye affinities have been described by Thom ('01, '01 a) and were the subject of many other studies (Kraus, '14; Guizzetti, '25, '27, '28). According to Okamoto ('30) the cystic cavities of the intermediate lobe contain air in children and colloid

does not accumulate until later in life. This conception, however, is most probably based on observations of cysts from which the colloid material fell out in the process of preparation. The cysts may contain a few erythrocytes and desquamated epithelial cells some of which are baso- or eosinophilic. Fat droplets may also be found here either free in the colloid or in phagocytes (Benda, '32; Scheele, '29).

As can be seen from this brief outline of the relevant literature there is no agreement between the various authors concerning the origin and significance of the colloid material. This uncertainty may, at least in part, be ascribed to the fact that the problem has, almost exclusively, been investigated by merely observing normal histological material without any effort to use an experimental approach. In the course of investigations concerning the morphological changes induced by the administration of various hypertonic solutions we observed incidentally that in rats receiving large doses of hypertonic sodium chloride solutions by intravenous infusion, the posterior lobe becomes extremely prominent because the hypophyseal cleft is distended with a large amount of fluid. Under these conditions there appears to be an increased secretion into the pituitary cleft. It was felt that with the aid of such an efficient method to stimulate the secretion of colloid we may be in the position to approach the problem of its secretion experimentally and thus learn more about the mechanism of colloid production.

In our first experiment ten male albino rats weighing 115-140 gm. were used. Under ether anesthesia 6 cc. of a 5% NaCl solution was slowly infused into the jugular vein of each animal. Approximately 10 minutes were necessary for each infusion because if such large amounts of hypertonic solution are more rapidly administered death may ensue during the injection period. All animals were killed 6 hours after the infusion. Mere naked eye inspection sufficed to show that the pituitary was greatly enlarged in all cases. Its borders were rounded and the capsule appeared to be under great tension. The posterior lobe was clearly detached from the anterior by

a greatly distended cyst-like cleft which was filled with clear colorless fluid. The anterior lobe appeared practically normal when viewed macroscopically, but its reddish color suggested pronounced hyperemia.

Histological sections of these pituitaries stained by the method described in a previous publication (Selye and McKeown, '35), revealed that the greatly distended cleft was filled by a rather thin type of colloid which, in the fixed state, appeared to be granular. All three lobes of the pituitary were very hyperemic and this was particularly true of the two so-called "lateral areas" in the anterior lobe (figs. 1 and 2). Degeneration of anterior lobe cells was especially widespread in these regions. However, throughout the tissue of the anterior lobe a large number of basophil cells was in the process of degeneration. In the early stages this change was characterized by a swelling of the cytoplasm which eventually led to a complete degeneration of the cell and transformation of the cytoplasm into a basophilic granular mass similar in appearance and staining reactions to the dilute granular colloid which distended the cleft. It was particularly striking that the nucleus was often still very well maintained at a time when the cytoplasm had already disintegrated. The cells immediately surrounding the small colloid accumulation resulting from the degeneration of a basophil, were then arranged in a manner resembling a lining epithelium. All intermediate stages between such minute cysts and large cystic cavities could be detected throughout the anterior lobes (figs. 3 and 4). Some of these cysts communicated with the main pituitary cleft and appeared to empty their contents into it. Within the colloid of the cleft, desquamated and partly degenerating anterior lobe cells, many of them still retaining the morphological characteristics of basophils, were readily distinguishable. The sinusoids in the anterior and middle lobe tissue immediately bordering upon the cleft were so greatly dilated that their walls protruded deeply into the cleft cavity. In two of the ten animals used in this series the cleft was

filled with blood, a fact which we are tempted to ascribe to the rupture of such engorged vessels.

It appeared of some interest to establish whether isotonic saline solutions would have the same effect. Hence a second experiment was performed on ten male albino rats weighing 120–141 gm. These received injections of as much as 10 cc. of an 0.9% NaCl solution into the jugular vein under circumstances similar to those in the first experiment. At autopsy, 6 hours after the injection, the pituitaries appeared macroscopically normal and histological examination likewise failed to reveal any conspicuous changes such as were seen after treatment with the hypertonic NaCl solution. It must be emphasized, however, that the physiological saline was much more readily excreted through the kidneys than the concentrated solution.

In order to determine whether an inhibition of the renal elimination of intravenously administered hypertonic saline would further intensify the hypophyseal changes caused by the latter, a third experiment was performed on ten male, adult, albino rats weighing 170–178 gm. These were bilaterally nephrectomized and immediately after this operation intravenously injected with 6 cc. of 5% NaCl in the same manner as the animals of the first experiment. They were sacrificed 6 hours after the injection and in these the pituitary changes were even more severe than in the similarly injected intact rats described above. We may therefore conclude that the hypophyseal reaction is not correlated with the actual process of the renal elimination of hypertonic solutions. On the contrary, it is intensified if such elimination is prevented, and hence the osmotic equilibrium of the body becomes more persistently deranged.

In the last experiment we used a vital dye in an attempt to gain further evidence concerning the mechanism of colloid secretion. Five intact and five nephrectomized, male, albino rats weighing 117–136 gm. were intravenously injected with 6 cc. each of an aqueous solution containing 5% NaCl and 0.5% trypan blue. Six hours after the injection all animals

were killed and it was found that while most of the tissues, especially in the nephrectomized rats, were slightly tinged by the blue dye, the cleft of the pituitary contained a particularly large amount of it so that the anterior lobe on the one side and the middle and posterior lobes on the other side were separated by a broad blue dark line (fig. 5). This experiment gives further support to the view that the large amount of fluid seen in the cleft at autopsy had actually been secreted during the few hours after the injection had been given.

From these observations it would appear most likely that the colloid in the anterior lobe cysts, as well as that seen in the cleft itself, is produced by the basophils of the anterior lobe. It arises, at least in part, by a process of holocrine secretion through the disintegration of the basophil cells themselves. In connection with this interpretation it should be mentioned that Ellison and Wolfe ('34) pointed out that in the pituitaries of spayed rats a good deal of colloid tends to accumulate in the cleft, and that this material is similar in its staining reactions to the granules found in the castration cells themselves. With regard to the physiological interpretation of our experiments little can be said until further work demonstrates whether hypertonic solutions of other compounds such as glucose, urea, etc., exert a similar effect. Such experiments are now under way but in the meantime our findings can only be regarded as a lead indicating the existence of certain interrelations between the basophils of the pituitary and the maintenance of the normal NaCl equilibrium or osmotic pressure in the tissues of the body.

SUMMARY

Experiments in the rat indicate that intravenous administration of hypertonic sodium chloride solutions causes swelling and eventually degenerative changes in the basophiles of the anterior lobe. Through confluence of the colloid material formed from the body of such basophils small cystic cavities arise throughout the glandular tissue. These cavities, as well as the hypophyseal cleft between the middle and anterior lobe,

gradually increase in size partly through a holocrine secretion of degenerating basophil cell debris and perhaps partly also through a merocrine secretion of the latter and filtration through the greatly engorged anterior lobe sinusoids. Eventually the colloid accumulation, especially in the cleft itself, takes such proportions that the posterior and middle lobes are separated from the anterior lobe by a wide cystic cavity. In its histochemical properties (staining, granulation etc.) the colloid in the cleft resembles that found in the smaller anterior lobe cysts and in the body of individual degenerating basophils.

The observations are interpreted as an indication of a close correlation between anterior lobe cysts and the pituitary cleft. It is believed, furthermore, that the basophils of the anterior lobe are involved in the formation of colloid in both these locations. Since this process of colloid formation is readily and rapidly stimulated by hypertonic salt infusions and not by equivalent amounts of isotonic solutions, it appears possible that the pituitary changes represent a reaction to derangements in the osmotic equilibrium of the body.

ACKNOWLEDGMENTS

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PLATE

PLATE 1

EXPLANATION OF FIGURES

1 Section through the pituitary of a normal control rat. Note slit-like cleft between middle and anterior lobe.

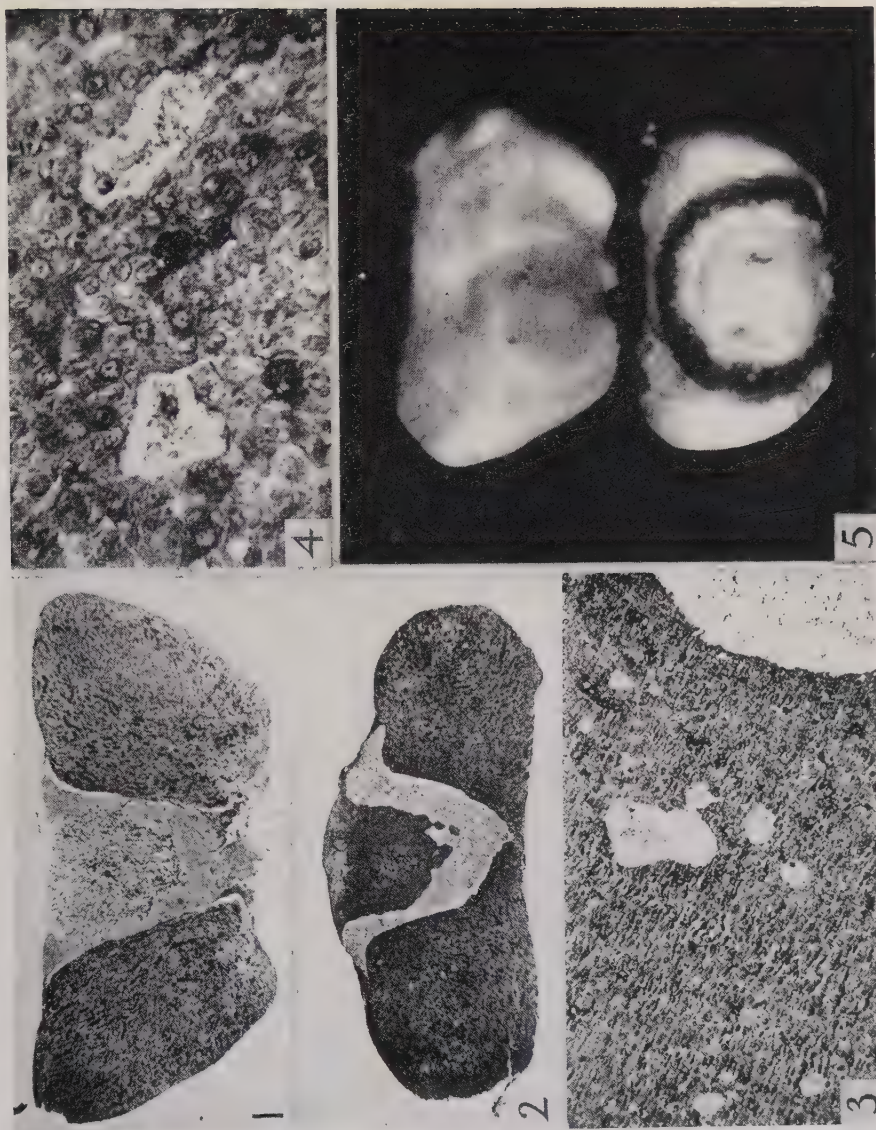
2 Section through the pituitary of a rat 6 hours after the administration of hypertonic NaCl solution. Note marked distention of the pituitary cleft by colloid (degree of distention is even greater during life as dehydration occurs in process of fixation). Small light spots throughout the anterior lobe tissue correspond to partly liquefied basophils. Hyperemia and degeneration are most pronounced in the two "lateral areas" of the anterior lobe.

3 Part of anterior lobe and cleft of another rat treated with hypertonic NaCl. Note that the small cysts in anterior lobe tissue and the cleft contain the same type of slightly granular colloid.

4 Two large degenerating basophils in the anterior lobe of a rat treated with hypertonic NaCl. Note that although cytoplasm has completely disintegrated the nucleus is still well preserved.

5 Naked eye view of normal pituitary (top) and that of a rat which had received hypertonic NaCl with addition of trypan blue (bottom). Note the presence of vital dye in the greatly distended cleft.

PITUITARY COLLOID SECRETION
HANS SELYE



REVIEWS OF APPROVED FILMS IN ANATOMY AND BIOLOGY

The following films in biology have been approved by one or more members of the Committees of the American Association of Anatomists or the American Society of Zoologists, and The Wistar Institute Committee.

Committee of the American Association of Anatomists:

Eliot R. Clark, Warren H. Lewis, Carl C. Speidel, and consultants.

Committee of the American Society of Zoologists:

Ralph Buchsbaum, Robert Chambers, Oscar W. Richards, and consultants.

Committee of The Wistar Institute of Anatomy and Biology:

Edmond J. Farris, Cranford Hutchinson, John S. Nicholas, and others as appointed.

THE BIOLOGY OF REPRODUCTION IN RATS

EDMOND J. FARRIS

(400 feet, kodachrome, silent)

By obtaining concurrent estrus and hour-by-hour running records in rats, extending from puberty to old age, Doctor Farris has discovered a "running" pattern which enables him to detect precisely, from the "running" record alone, the exact time of commencement of estrus and the time of many of the other reproductive incidents. The interesting story is told in a kodachrome film. The early scenes show the rat running on an inclined table, and the apparatus room in which cyclometer figures are tallied and photographed automatically at hourly intervals. Next are presented the varying mating behaviors, male and female, at different stages of estrus. Finally are shown living ovaries, oviducts and uterus at various stages, and the activities of the spermatozoa in the several parts of the female genital tract at different phases of estrus. The film should be of interest to all concerned with problems of reproduction in the rat, especially since it presents a refined method for ensuring and timing successful matings.

Since the female rat has a definite running urge, with peaks during estrus, it occurs to this reviewer that the search for a possible chemical factor that may be responsible might be stimulated by the introduction of a new name for such a hypothetical substance, and he offers the term "hormodrome" (from the Greek ὁρμάω [hormáo] I urge on (cf. hormone) and δρόμος [dromos] — a running).

Nicely timed and spaced; legends are well done; the film tells interestingly several different but correlated stories. It is a particularly worthwhile film because it shows strikingly several new methods and observations, which make it a real contribution.

★A — E. R. CLARK.

THIAMIN (VITAMIN B¹) DEFICIENCY IN THE CAT

DIETRICH C. SMITH AND GUY EVERETT

(16 mm., kodachrome, silent, 300 feet)

This film shows the effects on two cats of a thiamin-deficient diet, and the rapid recovery following administration of thiamin. For comparison four simple behavior types are selected: (1) walking, (2) righting reaction, (3) pupillary constriction and (4) eating. The cats are first shown in normal condition. Next they are seen following 3 weeks of a thiamin-deficient diet. The pronounced slowing up of and disturbance to neuromuscular reactions — the muscular weakness, loss of righting reaction, failure of pupillary reflex to light and convulsive seizures from slight stimuli — give a vivid picture of the results of thiamin deficiency. Each cat is then given a single injection of 1 mg. of thiamin, one intra-muscularly, the other intraperitoneally. Slight improvement is shown after 40 minutes, while 24 hours later both cats are almost normal.

The film gives a startlingly graphic, unforgettable picture of the importance of thiamin in the diet, and should be of great value in spreading knowledge of this essential vitamin. E — E. R. CLARK.

DIGESTION OF FOODS

A. J. CARLSON AND H. G. SWANN

(16 mm., monochrome, sound, 400 feet)

This is a superb picture giving, very largely by pictures from life but with supplementary animation, a resumé of food digestion. It shows mastication of food, flow of saliva and its increase by nerve and by acid stimulation; action of saliva on starch suspension; esophageal movements from life and by animation; stomach movements; action of gastric juice on coagulated egg albumin in a glass tube; a diagrammatic presentation of the hormonal action of substances, absorbed from the duodenum, on liver and pancreas; action of pancreatic juice on cream in a glass container; peristalsis in the small intestine; an all too short picture of contractions of villi; a diagram of absorption of fat via lymphatics and of other substance via blood-vessels; and contraction of the large intestine. The only criticism is that, for anatomical accuracy, the central lacteal is represented with too many branches. It usually runs as a single or double wide capillary at the center of the villus, and does not have side branches to the basal ends of the epithelial cells.

This reviewer has the greatest admiration for the ingenuity and skill which has been manifested in the production of this admirable picture. It is amazing that so much can be shown so effectively in so short a time. B — E. R. CLARK.

★ Copy deposited in the Film Depository of The Wistar Institute, and is available to all scientists for use at The Wistar Institute.

A — Available for sale or rent by The Wistar Institute.

B — Available for sale or rent by the author or distributor.

C — Not available for sale or rent.

D — For information, communicate with the author.

E — For information, communicate with The Wistar Institute.

THE ORIGIN OF THE VERTEBRAL COLUMN IN THE DEER-MOUSE, *PEROMYSCUS MANICULATUS* RUFINUS¹

E. CARL SENSENIG

Department of Anatomy, University of Alabama

TWO PLATES (SEVEN FIGURES)

INTRODUCTION

The embryological development of the vertebral column has been intensively investigated by Froriep (1886), Weiss ('01), Dawes ('30), von Bochmann ('37), and others, all of whom, however, restricted their studies to a specified region of the column. Froriep (1886), Weiss ('01), Barge ('18), and Hayek ('23) were primarily concerned with the segmentation of the chordal part of the skull and secondarily with the anterior cervical vertebrae. Dawes ('30) considered the cervical region and included a discussion of the development of the thoracic region. Von Bochmann ('37) investigated the caudal and lumbosacral segments. These investigations contain conflicting opinions. To clarify their findings, a study of the development of the entire vertebral column in some mammal is needed. This investigation was undertaken with that object in view. A preliminary report of the results already has appeared in abstract (Sensenig, '42).

The embryological development of the vertebral column is generally divided into three periods: blastemal, cartilaginous and osseous. To determine the origin of any vertebral element it is necessary to trace it back to the blastemal stage, as here all of the vertebral primordia arise. Within the

¹ A condensation of a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, in the University of Michigan.

blastemal period development is very rapid and consequently a closely graded series of stages is necessary accurately to determine the origin and formation of a given structure. It is with that period that this paper is primarily concerned. The cartilaginous and osseous periods have been critically studied by many authors and therefore will receive only secondary attention.

ACKNOWLEDGMENTS

I am indebted to Dr. A. H. Stockard, University of Michigan, for the supervision of the work related to this problem, and to Dr. L. R. Dice who supplied the initial stocks and breeding space in the Laboratory of Vertebrate Genetics, University of Michigan. I also wish to express my gratitude to Dr. C. M. Goss, University of Alabama, for his helpful criticisms of this paper and to Dr. E. A. Hunt for the construction of the diagrams.

MATERIALS AND METHODS

The prairie deer-mouse, *Peromyscus maniculatus rufinus*, was used in this study. The embryos were removed from the uterus, measured for crown-rump length, and placed in the fixing fluid as rapidly as possible. Zenker's fluid was used for embryos of 19 days or younger, but because of its more rapid rate of penetration Bouin's fluid was used for older embryos. Following fixation the embryos were run through dioxane, embedded in paraffin, sectioned at 10 micra and stained in a modified Mallory's triple stain in which alum cochineal was substituted for fuchsin.

The material used consisted of transverse, frontal, and sagittal serial sections of embryos having a crown-rump length of 2.0, 3.0, 4.0, 4.8, 6.2, 7.0, 8.6, 9.0, 10.0, and 12.0 mm. and with gestation periods of from 10 to 20 days.

OBSERVATIONS

Mesoblastic somites. Each mesoblastic somite is composed of a dermatome, myotome and sclerotome, the sclerotome forming its mesial portion. The sclerotomes produce the post-

otic axial skeleton. Each sclerotome is partially divided by a fissure into a cranial and a caudal sclerotomite. Distinct sclerotomites appear in the sclerotomes of the last two occipital and all succeeding segments.

Sclerotomic fissure. In *Peromyscus* the sclerotomites in all regions of the vertebral column are separated by a sclerotomic fissure. Its presence in the occipital region of a mammal is here observed for the first time, it being present in the fifth occipital segment of the mouse. The fissure forms after the disappearance of the myocoele and is therefore never continuous with that cavity. It is present in the posterior cervical and anterior thoracic regions of 2.0-mm. embryos and in all regions of the vertebral column in 3.0-mm. embryos. It has disappeared in the fifth occipital and in the anterior cervical segments of 4.0-mm. embryos, and in the 4.8-mm. specimen it has vanished in all but the caudal segments.

Resegmentation of the sclerotomes. It is functionally necessary that a given vertebra occupy an intersegmental position in relation to the muscles which act upon it. The original vertebral segment or sclerotome occupies an intrasegmental position opposite its myotome, and a consequent resegmentation of its components is required. The cranial sclerotomite of one sclerotome joins the caudal sclerotomite of the preceding sclerotome. In the provertebral segment thus formed the cranial and caudal sclerotomites of the original somite have reversed their position. The definitive vertebra is therefore composed of two halves, each derived from a different somite, an arrangement which permits the proper function of the axial musculature. As a result of this resegmentation the cranial sclerotomite of the first cervical somite is set free, there being no caudal sclerotomic component anterior to it. At the caudal end of the vertebral column a caudal sclerotomite is similarly freed because there is no cranial sclerotomic component posterior to it. The freed cranial sclerotomite contributes to the proatlas while the posterior one degenerates.

Notochord. The notochord first appears as a slender non-constricted rod extending from the infundibular region to the tip of the tail. A migration of very diffuse sclerotomic tissue forms a network of cells surrounding the notochord. This is the perichordal tube, which takes part in the future development of the notochord (figs. 1 and 3). A thin, discontinuous, fibrous sheath, through which sclerotomic cells migrate, surrounds the notochord in all but the more distal caudal segments. Migration of sclerotomic cells through this sheath is greatest in the region of the perichordal tube (figs. 1, 3 and 4). As development progresses the fibrous sheath becomes continuous and thicker and is extended into the more distal caudal segments. Intersegmental waves appear in the fibrous sheath opposite the posterior cervical and thoracic regions of the 3.0-mm. embryos and soon are present in the remaining trunk regions, but they never form in the tail. The dorsal crests of these waves lie between the rudiments of the centra, and their ventral furrows lie within the rudiments of the centra. The waves disappear before the appearance of cartilage. Notochordal dilations and constrictions first appear in the 6.2-mm. embryos and develop rapidly, until in 12.0-mm. embryos the notochord has completely vanished within the centra and has greatly expanded in the intervertebral discs between the centra. In the thoracic region these expanded zones reach a diameter of 185 micra and are filled with large, strongly vacuolated cells. Each expanded zone forms a nucleus pulposus, which is derived from both the original notochordal cells and the sclerotomic tissue which entered the notochord through gaps in its sheaths. The only functional remnants of the notochord found in the adult are those in the nuclei pulposi and the apical ligament of the dens.

Mesial sclerotomic migration. Each caudal sclerotomite is composed entirely of densely cellular tissue, except for a small posteromesial area of loosely cellular tissue (figs. 1 and 4). Each cranial sclerotomite is densely cellular laterally and posteromesially, while the remainder of it is diffuse and is continuous with the small, loosely cellular, posteromesial

area of the caudal sclerotomite mentioned above (figs. 1 and 4). The diffuse regions form the primary centra, while the mesial portions of the dense regions form the perichordal discs and their lateral portions form the neural arches and ribs. The perichordal disc is divided transversely into three parts. The anterior part and the posterior part unite with the adjacent primary centrum to produce the definitive centrum. The remaining middle portion forms the greater part of the definitive intervertebral disc. Each vertebra, therefore, is composed of contributions from the two adjacent sclerotomites derived from two different sclerotomes (figs. 1 and 4).

Neural arches. The neural processes are formed from cells which migrate dorsad from the lateral portion of the caudal sclerotomite, while the transverse processes form later as lateral outgrowths from these processes (figs. 3, 4 and 5). To complete the neural arch each pair of neural processes is connected above the neural tube by a supraneural strand of cells which chondrify late in development. A mesial migration of cells from the dorsolateral portion of the caudal sclerotomite forms an epichordal strand which connects each pair of neural arches ventral to the neural tube (figs. 3 and 6). Chondrification appears first in the more ventral part of the neural processes (pedicles), extends rapidly throughout the arches and the epichordal connecting strand, and finally invades the transverse processes.

Articular processes. The articular processes or zygapophyses are formed from cells which migrate dorsad from the dorsolateral part of the cranial sclerotomite (figs. 2 and 5). These cells first form a mass situated midway between two adjacent arches. By multiplication they gradually extend cranially and caudally to form a bridge of cells connecting the two arches. Within this bridge the pre- and postzygapophyses and the zygapophyseal articulations form.

Ribs. The ribs arise from cells which migrate from the posterolateral region of the caudal sclerotomites and from the anterolateral region of the cranial sclerotomites into the

myosepta (figs. 1, 3 and 4), which are intersegmental, lying between adjacent myotomes, but are opposite the definitive vertebrae. Through continued cellular migration from these sources, the rib extends ventrad in the myoseptum. A mesial migration from the ventrolateral portion of the caudal sclerotomite forms a subchordal strand of cells connecting the two rudiments of a pair of ribs (fig. 3).

Occipital region. The occipital region is composed of five segments plus a small mass of undifferentiated tissue anterior to the first distinct occipital segment, which probably is the vestige of a segment anterior to the first. The sclerotome of the fifth or most posterior of these segments is as well developed as is that of a typical vertebral segment, but the size and the degree of differentiation of the occipital segments decrease rostrally (fig. 7). Neural processes form only in the fifth occipital segment, where they give rise to the exoccipital bones. The fourth and fifth occipital sclerotomes are divided into sclerotomites, though a fissure separated only those of the fifth segment. Arising from all of the occipital segments is a mesial migration of cells which surround the notochord and give rise to the basioccipital bone.

Proatlantal half-segment. The proatlantal rudiments are formed from the cranial sclerotomite, freed from the first cervical segment during resegmentation, previously indicated. The rudiment of its centrum forms the tip of the odontoid process and its epichordal strand becomes the transverse ligament of the atlas. Its neural processes and subchordal strand join the corresponding rudiments of the atlas. Thus the odontoid process is formed of the proatlantal centrum, the atlantal intervertebral disc and centrum, and the axial intervertebral disc, which also join the centrum of the axis.

DISCUSSION

Notochord. The segmental waves, indicated by Carrier (1890) but first described by Minot ('07), are present in all vertebral regions but the tail of *Peromyscus*. Wherever the waves appear their dorsal crests lie between and their ventral

furrows lie within the vertebrae. This agrees with the observations of Minot ('07) in the cat, dog and rabbit but opposes those of Dawes ('30) in the albino mouse and of Minot in the pig. These segmental waves, which Dawes states "persist until chondrification is well advanced", have become less evident in 6.2-mm. *Peromyscus* embryos and are no longer visible at the onset of chondrification.

Constrictions and dilations in the notochord of *Peromyscus* first appear in 6.2-mm. embryos. In development they resemble those described by Dawes ('30) for the albino mouse, but in *Peromyscus* they appear at a slightly later stage than in the albino mouse. The notochord becomes constricted at the ventral furrows and dilated at the dorsal crests of the waves.

The origin of the nucleus pulposus, which forms at the notochordal dilations, has been the subject of considerable discussion. Two opposing theories have been published. Luschka (1858) believed it to be of notochordal origin, while Virchow (1857) believed it to arise through liquefaction of the cells of the intervertebral disc outside of the notochord. Williams ('08) reported the mammalian nucleus pulposus to be of notochordal origin, forming through a strong vacuolization of the notochordal cells and not by extrachordal liquefaction of the intervertebral disc. Gadow (1896) doubted that it was formed from either structure alone, but suggested that both the notochord and the intervertebral disc might contribute to it.

In *Peromyscus*, although the volume of the notochordal cells in the intervertebral disc is greatly increased by vacuolization, migration of mesenchymal cells from the perichordal discs into the notochord through its sheaths was observed. This opposes Virchow's view of a liquefaction of extrachordal intervertebral tissue and indicates that sclerotomic cells from the perichordal disc enter the notochord and contribute to the formation of the nucleus pulposus. Both notochordal tissue and intervertebral disc tissue are clearly distinguishable through all stages in *Peromyscus*.

Sclerotomes. A considerable discussion has arisen over the existence of a sclerotomic fissure in mammals. Froriep (1886) failed to observe it in the embryos of *Bos*. Accordingly, Gadow and Abbott (1895) failed to grant the probability of its existence. In mammals it was first observed by Weiss ('01) in the albino rat, and later by Bardeen ('05) in man, and by Dawes ('30) and von Bochmann ('37) in *Mus*.

The sclerotomic fissure is not continuous with the myocoele in *Peromyscus*, as Schauinsland ('06) found it in *Sphenodon*, but forms after the disappearance of the myocoele. In *Mus* von Bochmann ('37) found a remnant of the fissure in the caudal vertebrae after chondrification had begun. This is not true in *Peromyscus*, where the fissure vanishes prior to the onset of chondrification.

The final proof of the existence of a sclerotomic fissure gave support to Remak's (1855) theory of the "Neugliederung der Wirbelsäule," the rearrangement of the vertebral column. Because of his failure to identify the sclerotomic fissure Froriep (1886) refused to accept Remak's theory, as also did Gadow and Abbott (1895). However, in a later publication Gadow ('33) rearranges his scheme and accepts Remak's theory, as do Weiss ('01), von Bochmann ('37), and the present author, as well as the majority of investigators of non-mammalian vertebrae.

The terminology introduced by Gadow (1895), basidorsal and basiventral for the dorsal and ventral components of the caudal sclerotomite, and interdorsal and interventral for the corresponding components of the cranial sclerotomite, is not applicable in *Peromyscus*. Dawes ('30) divided the caudal sclerotomite in the albino mouse into dorsal and ventral halves on the basis of cell orientation and density. No such difference occurs in *Peromyscus*. In this animal only an arbitrary division of the sclerotomites into dorsal and ventral halves can be made, by regarding that area of the sclerotomite above the level of the notochord as dorsal and that area below the notochord as ventral. In *Peromyscus*, and in *Mus* according to von Bochmann ('37), the sclerotome is a continuous homo-

geneous structure from dorsal to ventral tips and is not differentiated into distinct dorsal and ventral components as has been reported in the lower vertebrates.

In *Peromyscus* and in *Mus*, as observed by Remane ('36) and von Bochmann ('37), densely cellular tissue is not restricted to the caudal sclerotomite, observations which are contrary to those of Weiss ('01), Bardeen ('05), Dawes ('30), and Goodrich ('30). Except for a small posteromesial portion, densely cellular tissue appears throughout the caudal sclerotomite and is continuous laterally, adjacent to the myotome, with the dense tissue of the cranial sclerotomite. Furthermore, the two sclerotomites are continuous by dense tissue in the perichordal tube and in the epichordal and subchordal strands, though the density of these connecting structures varies in the two sclerotomites (figs. 2 and 3).

Early authors reported that the intersegmental vessels and sclerotomic fissures accurately delimit the sclerotomites. These structures are only partial boundary markers, the sclerotomic tissue being continuous around them (Remane ('36), von Bochmann ('37) and the present author). They lie near but do not coincide with the limits of the sclerotomites. Remane ('36) reported that under the influence of strong cellular masses intersegmental vessels may migrate from their original position and cause misinterpretation when used as boundary marks.

The contribution of all parts of a sclerotome to a mammalian vertebra makes it impossible to accept Gadow's classification of them as gastrocentrous. According to Gadow a gastrocentrous vertebra is formed predominantly from the basidorsal and interventral elements, the basiventral element is suppressed to fibrocartilage and a small intercentrum or chevron bone, and the interdorsal is absent. Components from all four regions of the sclerotome — basidorsal, basiventral, interdorsal and interventral — are present in the vertebrae of *Peromyscus*, which, therefore, are not gastrocentrous, and do not fit into Gadow's classification.

Occipital region. The occipital region in *Peromyscus* corresponds closely to that in *Mus* as described by Dawes ('30). In 4.0-mm. and 4.8-mm. embryos there are five occipital nerve roots, one in each of the five segments; and there is an inter-segmental artery at the anterior border of each segment. Dawes ('30) did not find an occipital nerve root in the first of the five segments in *Mus*. Anterior to the first segment in *Peromyscus* there is a mass of unsegmented mesoderm which may be the remnant of one or more somites. De Beer ('37) believes the most anterior segment of Dawes to be the second metotic somite. If this interpretation can be accepted in *Peromyscus*, then the undifferentiated mesoderm anterior to it can be the degenerate first metotic somite and the total number was six. Froriep (1886) found only four segments in the occipital region of *Bos*, a condition due perhaps to the suppression of the second metotic segment and its inclusion in unsegmented mesoderm with the first metotic segment.

The sclerotomites of the fifth occipital segment of *Peromyscus* are partly separated in 4.0 and 4.8 mm. embryos by a sclerotomic fissure. This fissure is transitory, which may account for the fact that Dawes ('30) failed to find it in *Mus*. Both of the sclerotomites of the fifth occipital segment yield strong cell migrations mesiad around the notochord and dorsad on either side of the neural tube. Within this tissue the posterior part of the basioccipital and the exoccipital bones are formed. From the four anterior segments cells migrate mesiad only around the notochord, and within them forms the anterior mass of the basioccipital bone. The manner of formation of the three occipital bones agrees with the findings of Dawes ('30) in *Mus*.

Proatlas, atlas and axis. The structure of the first two cervical vertebrae varies considerably from that of the remaining cervical and trunk vertebrae. These variations are related to the functions of articulation with and the pivotal rotation of the skull. Likewise, the developmental history of the axis and atlas is strikingly different from that of the other vertebrae.

The proatlantal half-segment, in contrast to a typical cranial sclerotomite, forms a strong subchordal strand, and a strong neural arch rudiment, which pass directly into the corresponding components of the caudal sclerotomite of the atlas. Cells also migrate mesiad from it and surround the notochord. The suboccipital or first cervical nerve lies within the proatlantal half-segment. Barge ('18) found the same condition in sheep embryos, where the proatlantal arch and subchordal strand (or ventral arch) fuse with those of the atlas. The proatlantal centrum, formed by the migration of cells around the notochord, gives rise to the tip of the odontoid process of the epistropheus as reported by Hayek ('23) and Gadow ('33).

The rostral portion of both the ventral and dorsal arches of the atlas are formed from the proatlantal half-segment. The remainder of each of these arches is derived from the neural arch and the subchordal strand of the caudal sclerotomite of the atlas. The development of the atlantal arches in *Peromyscus* closely resembles that in *Bos* as described by Froriep (1886). They chondrify early, as in *Mus* (Dawes, '30), but in *Peromyscus* the cartilage does not penetrate as deeply into the epichordal strand, giving rise instead to the transverse ligament of the atlas. The epichordal strand, "Vertikalplatte" of Weiss, is not present in the atlantal segment of the rat (Weiss, '01). The mesial migration around the notochord in the atlantal segment forms the atlantal centrum and intervertebral disc, which becomes incorporated into the dens of the epistropheus.

The development of the neural arch of the axis is similar to that of the neural arch of a typical vertebra, while its centrum forms the base and its intervertebral disc forms the proximal portion of the odontoid process. The formation of the axis and its odontoid process, as previously described, is in agreement with the report of Dawes ('30) for *Mus* but varies from that of Weiss ('01) for the albino rat, where he described a very poorly developed preatlantal intervertebral disc, his "Horizontalplatte."

Ribs. The majority of authors, including Goodrich ('30) and Dawes ('30), accept and state that the rib rudiment arises from the ventral part of the caudal sclerotomite, and is therefore from the basiventral element. My observations and those of von Bochmann ('37) show that the rib rudiment is derived from both sclerotomites though the greater portion of it does come from the caudal sclerotomite, therefore from both inter-ventral and basiventral components. In 4.0-mm. embryos cells are seen migrating from the dense lateral portion of the anterior region of the cranial sclerotomite and the posterior region of the caudal sclerotomite. These penetrate the myoseptum as the rib rudiment. The neck of the rib forms in the myoseptum just lateral to the sclerotomite, and through apical growth the rib extends ventrad into the myoseptum.

Dawes ('30) and Goodrich ('30) reported that the capitulum and tuberculum are derived entirely from the ventral part of the caudal sclerotomite, or basiventral. Von Bochmann ('37) accepted this view but reported that the anterior part of the capitulum is formed from the posterior part of the cranial sclerotomite of the same somite. The dense mesial cell migration in the posterior part of the cranial sclerotomite forming the anterior part of the perichordal disc. I found in *Peromyscus* that the anterior and posterior thirds of the perichordal disc become incorporated in the adjacent ends of the two centra and, that the intervertebral disc arises entirely from the middle third, that is, from the caudal sclerotomite. I found no evidence that the cranial sclerotomite contributes to the capitulum.

In *Peromyscus* the transverse process forms as an outgrowth of the neural arch, thus arising from the caudal sclerotomite. The tuberculum extends dorsad from the rib rudiment to become continuous with this process. With the formation of cartilage a strip of membranous tissue remains between the two processes. Within this strip appears a fissure, and in this fissure the diapophyseal joint cavity later develops, the tissue surrounding the fissure forming the synovial capsule. Similarly, a membranous strip separates the capitulum

from the intervertebral disc in which the parapophyseal articulation later develops. These observations agree with those of Dawes ('30) and von Bochmann ('37). However, they do not agree with the findings of Bardeen ('05), who reported that in man the tuberculum is formed by outward and downward growth of the transverse process which extends to make contact with the rib.

In the cervical region the rudiments of the costal and transverse processes are present. Both extend laterad and fuse at their lateral borders to form the costotransverse process.

SUMMARY

1. In all regions of the vertebral column and in the fifth occipital segment the sclerotomes are divided by sclerotomic fissures into cranial and caudal halves, the sclerotomites.

a. The sclerotomites are not completely separated by the sclerotomic fissures, nor are the sclerotomes completely separated by intersegmental vessels.

b. The sclerotomites are recombined to form the definitive vertebrae, in accordance with Remak's theory of "Neugliederung der Wirbelsäule."

2 a. In early embryos gaps are present in the sheaths of the notochord through which sclerotomic cells migrate into the notochord. These cells share with the notochordal cells in the formation of the nucleus pulposus.

b. Segmental waves are present in the notochord of 3.0 mm. embryos, their dorsal crests lying between the vertebrae and their ventral furrows within the vertebrae. They disappear before the onset of chondrification.

3. Densely cellular tissue is not restricted to the caudal sclerotomite but is also present throughout the lateral and posterior parts of the cranial sclerotomite.

a. A dorsal migration of cells from the caudal sclerotomite forms the neural arch and its transverse process. A similar cell migration from the cranial sclerotomite forms the articular processes.

b. A mesial, densely cellular migration, the greater part supplied by the caudal sclerotomite, forms the perichordal disc while a mesial, loosely cellular migration, the greater part supplied by the cranial sclerotomite, forms the primary centrum. The perichordal disc divides transversely into three portions. The mid-portion forms the intervertebral disc and the remaining portions become incorporated into the adjacent primary centra to form the definitive centra.

c. A lateral cell migration forms the rib. The cells originate from both the cranial and the caudal sclerotomite, not from the caudal sclerotomite (basiventral) only.

4. The occipital region is composed of five distinct segments, of which the fifth or most caudal has a well developed sclerotome with its sclerotomites separated by a fissure. An undifferentiated mass of tissue probably represents a degenerate segment anterior to the first.

5. The proatlantal half-segment is the cranial sclerotomite of the first cervical sclerotome and has no caudal sclerotomite associated with it. Its dorsal and ventral arches are incorporated into those of the atlas, its epichordal strand forms the transverse ligament of the atlas, and the rudiment of its centrum forms the tip of the dens. The centrum and intervertebral disc of the atlas and the intervertebral disc of the axis form the remainder of the dens.

6. The contribution from all four elements of the sclerotome to the mammalian vertebra precludes it from being correctly classified as gastrocentrous after Gadow's terminology.

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ABBREVIATIONS

a. p., articular process	n. p., neural process	sp. g., spinal ganglion
c., centrum (primary)	p. d., perichordal disc	sp. n., spinal nerve
ep. s., epichordal strand	p. t., perichordal tube	s. s., subchordal strand
i. a., intersegmental artery	r. r., rib rudiment	x-y, caudal sclerotomite
m., myotome	s. f., sclerotomic fissure	x-z, one segment
n., notochord	sp. c., spinal cord	y-z, cranial sclerotomite

PLATE 1

EXPLANATION OF FIGURES

Figures 1-3 are diagrammatic drawings.

- 1 Diagrammatic frontal section through the mid-thoracic region of a 4.5-mm. mouse embryo. $\times 200$.
- 2 Diagrammatic transverse section through a cranial sclerotomite in the mid-thoracic region of a 4.5-mm. embryo. $\times 50$.
- 3 Diagrammatic transverse section through a caudal sclerotomite in the mid-thoracic region. $\times 50$.

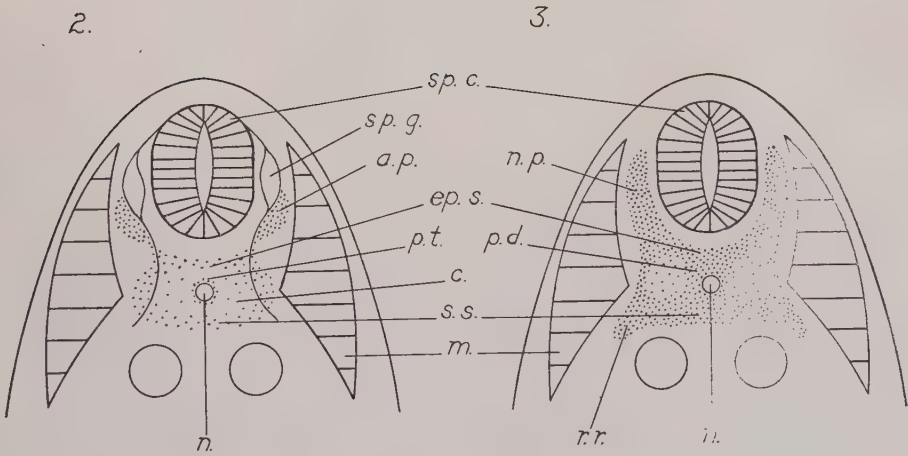
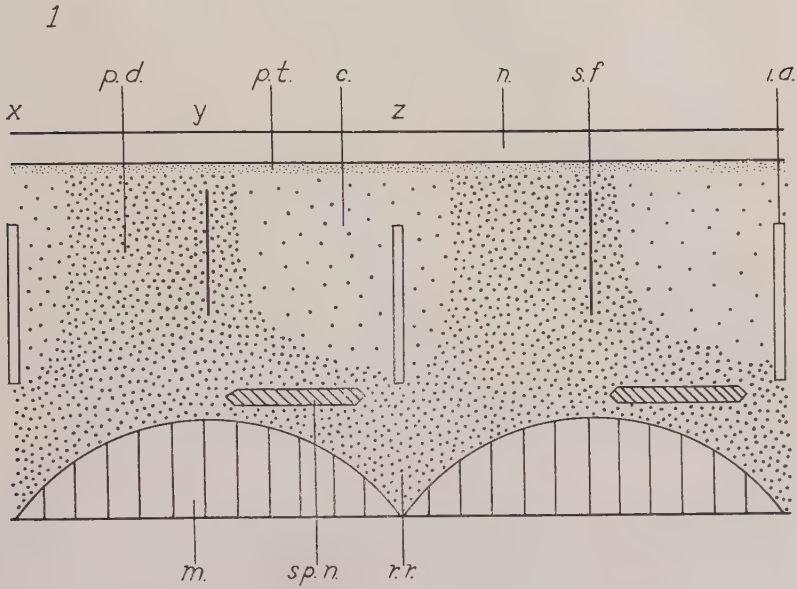


PLATE 2

EXPLANATION OF FIGURES

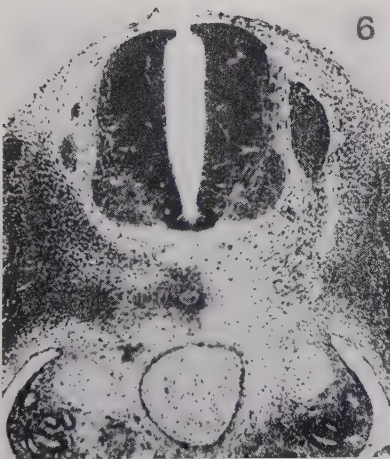
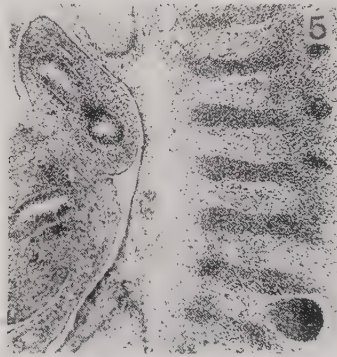
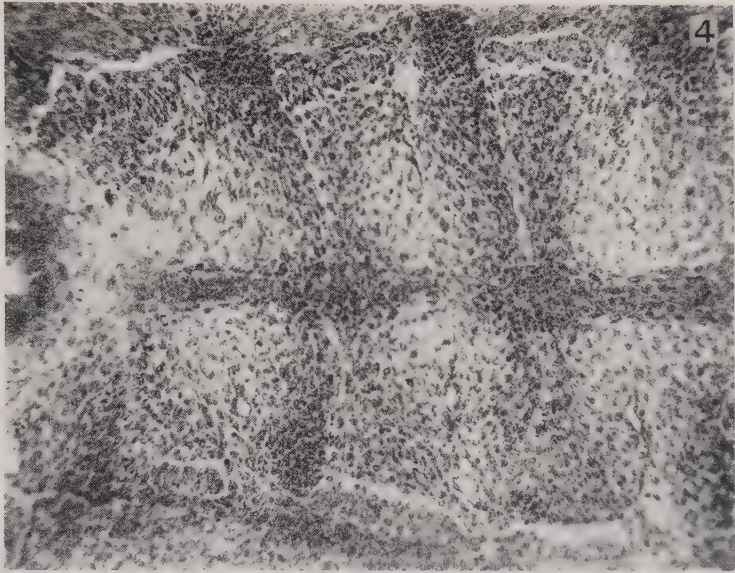
Figures 4-7 are photographs.

4 Frontal section through the notochord in the thoracic region of a 4.8-mm. embryo. Compare with figure 1. $\times 90$.

5 Parasagittal section through the neural and articular processes in the upper thoracic region of a 4.8-mm. embryo. $\times 50$.

6 Oblique transverse section through a cranial sclerotomite in the mid-thoracic region of a 4.8-mm. embryo. The left side is through the posterior densely cellular portion, while the right side is through the anterior loosely cellular portion of the cranial sclerotomite. $\times 50$.

7 Parasagittal section through the occipitocervical region of a 4.8-mm. embryo. Shows the neural processes of the fifth occipital and first three cervical vertebrae. $\times 50$.



ASSIMILATION OF AVIAN YOLK AND ALBUMEN UNDER NORMAL AND EXTREME INCUBATING TEMPERATURES

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SIX FIGURES

Yolk and albumen are the chief sources of food and water in avian embryonic development. Their disappearance from the egg is directly proportional to the metabolic activity and economic uses of these substances for the growth and development of the embryo. To know the rate of assimilation of yolk and albumen in the avian egg under optimum (see review of Romanoff and Romanoff, '33 on chicken egg) and adverse conditions would, therefore, be of both academic and practical interest.

METHODS AND MATERIALS

The eggs of seven different species of birds were used in this study. They were White Leghorn hens (*Gallus domesticus*), Ring-necked pheasants (*Phasianus torquatus*), Bobwhite quail (*Colinus virginianus*), White Holland turkeys (*Meleagris gallopavo*), Pekin and Runner ducks (*Anas domesticus*), and Mallard duck (*Anas boschas*). From two to four hundred eggs of each species were incubated in the laboratory incubators (Romanoff, '32). Conditions for normal development similar to those given in another paper (Romanoff, '43) were maintained.

In determining the effect of temperature on the assimilation of yolk and albumen, chicken eggs were used rather than eggs of the less common species. Normal temperature was 37.5°C.

The extreme temperatures were 34.5° and 39.5°C., and intermediate ones were 36.0° and 38.5°C. All other environmental factors were normal.

The dense yolk, liquefied yolk, and albumen of the egg were separated after coagulation of the entire egg in boiling water (Romanoff, '32 a). Two to six eggs of each species were dissected at 24-hour intervals and the separate parts were weighed.

EXPERIMENTAL RESULTS

Normal conditions

The data for all seven species showed similar trends in the assimilation of yolk and albumen during embryonic development. The dense yolk (fig. 1) maintained a fairly constant weight level for nearly three quarters of the incubation period. During the first week of incubation, after some gain, the weight dropped slightly, as observed previously in fertile and infertile chicken eggs (Romanoff and Romanoff, '33; Romanoff, '40). The greatest decrease in yolk occurred close to hatching time, constituting not more than one-third of its original weight.

On the other hand, the liquefied (thin) yolk (fig. 2) appeared only after a few days of incubation. It increased rapidly, reaching a peak at about one-third of the developmental period, then decreased sharply and disappeared near the mid-period.

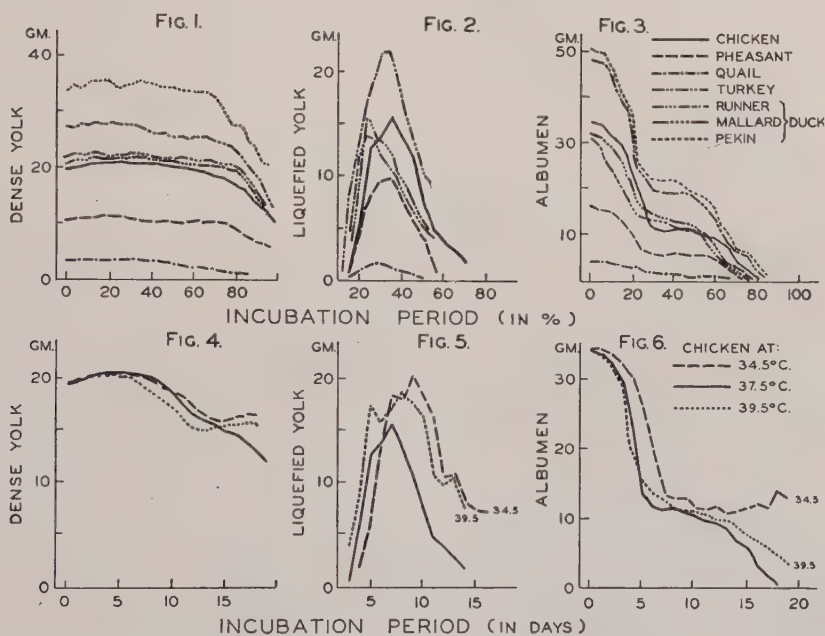
The time of rapid increase of liquefied yolk coincided with the rapid decrease of albumen (fig. 3). Then the albumen reached a certain level and finally dropped to nearly zero just a few days before hatching time.

Extreme temperatures

Extreme temperatures, 34.5° and 39.5°C., did not markedly affect the weight of dense yolk of chicken eggs during most of the incubation period, but at hatching much less of the yolk had been assimilated than under normal temperature, 37.5°C. (fig. 4). Under both extreme temperatures the amount of lique-

fied yolk was also much greater at the peak and it was present longer than under normal conditions (fig. 5).

The initial rate of assimilation of albumen (fig. 6) was somewhat retarded by low temperature. At hatching it could be accelerated by high temperature, as was shown previously



Figs. 1 to 6 Assimilation of yolk and albumen in the developing avian egg.

The curves in figures 1, 2 and 3 show assimilation of yolk and albumen, and appearance of liquefied yolk. Abcissae represent the per cent of time for the full embryonic developmental period — chicken 20 days, pheasant and quail 23 days, and turkey and duck 27 days.

The curves in figures 4, 5 and 6 show assimilation of yolk and albumen of the chicken egg under extreme temperatures.

by Romanoff, Smith and Sullivan ('38). However, a considerable amount of albumen was still found in the egg, especially at low temperature.

Intermediate temperatures, 36.0° and 38.5°C., did not affect noticeably the rate of assimilation of yolk or albumen, although tendencies toward similar temperature effects were present.

DISCUSSION

The yolk of the egg is rich in concentrates. The chicken egg yolk, for example, contains over 52% of dry matter. The albumen, on the other hand, contains about 88% of water (Romanoff, '30). Consequently, during incubation, with a considerable movement of water in various parts of the egg, the yolk is more stable than the albumen.

The chief role of the yolk is obviously to furnish food for the embryo, while the role of the albumen is to furnish water for the formation of allantoic and amniotic fluids (Romanoff, '43), and later for the embryo. However, the water from the albumen is first transferred to the liquefied yolk, which is nothing but an intermediary developmental structure of the egg. According to Shklyer ('37), its origin is apparently related to the activity of enzymes. With incubated infertile eggs there is no rapid decrease in albumen, but a gradual drop in volume, in spite of the transference of water to the yolk and evaporation through the shell (Romanoff, '40).

These quantitative changes in yolk and albumen of the developing egg are in agreement with the calculations of Needham ('31), which show that during the first week the embryo uses carbohydrates, in the second week, proteins (presumably from the albumen), and in the third week, fats (largely from the yolk).

The rate of absorption of yolk and albumen under normal conditions is closely related to the rate of growth of the embryo. Earlier work (Romanoff and Romanoff, '33) has shown that the decrease of dry weight of chicken yolk and albumen combined is directly proportional to the increase in weight of the embryo.

Under extreme temperature conditions the growth of the embryo is retarded (Romanoff, Smith and Sullivan, '38). Consequently, the assimilation of the yolk is slowed, and that of albumen is incomplete as indicated by the presence of both yolk and albumen at the time of hatching (cf. figs. 5 and 6).

SUMMARY

Under normal conditions the rate of assimilation of yolk and albumen is similar in all species of birds studied: chicken, turkey, pheasant, quail, and three species of ducks. The dense yolk shows little change until the latter part of incubation. At hatching it decreases to nearly one-third of its original weight. The liquefied (thin) yolk appears after a few days of incubation. Its appearance coincides with the rapid decrease in albumen. The liquefied yolk disappears rapidly about the mid-period of incubation. The albumen is completely assimilated a few days before hatching.

Both extremes of temperature, 34.5 and 39.5°C., modify the rate of assimilation of yolk and albumen. In chicken eggs the dense yolk is more slowly assimilated during the latter part of incubation. There is a greater accumulation and longer persistence of liquefied yolk. The albumen is slowly assimilated and a considerable amount of it is still found in the egg at the time of hatching.

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THE SUPERFICIAL HEPATIC BRANCHES OF THE VAGI AND THEIR DISTRIBUTION TO THE EXTRAHEPATIC BILIARY TRACT IN CERTAIN MAMMALS¹

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ONE FIGURE

An experimental investigation was undertaken for the purpose of obtaining further data on the innervation of the gallbladder by the vagi. Descriptions of the nerves to the extrahepatic biliary tract, presented in sufficient detail to be of value in the investigation, were not found, although several studies of the anatomy of these nerves have been made by Alexander ('40), Ellenberger and Baum (1891) and McCrea ('24). Accordingly it became necessary to precede the physiologic investigation by a study of the superficial hepatic branches of the vagi. As it was desired to use animals of three species (the dog, cat and guinea-pig) in the study, dissections of the nerves of these animals were made. After completion of the dissections and the physiologic investigation for which they were made, it appeared that these anatomic data might prove of value in other physiologic investigations.

MATERIALS AND METHODS

Unembalmed specimens of dogs, cats and guinea-pigs were used. The bodies of lean animals were selected because in such

¹ Abridgment of part of thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of M.S. in Experimental Surgery. Work done at the Institute of Experimental Medicine, Mayo Foundation. The writer wishes to express his indebtedness to Prof. F. C. Mann for constant help and guidance.

specimens the absence of adipose tissue in the lesser omentum and hepatic pedicle facilitated the identification of the nerves. A small depilatory forceps, cuticular scissors and a straight suture needle having a sharp cutting edge were found to make suitable dissecting instruments. The larger nerve trunks could be recognized with the naked eye but a magnifying lens or dissecting microscope was necessary for the dissection of the finer nerves. The plan of study was first to expose and identify the various arteries which provide the framework and serve as guides for the distribution of the nerves. Then the nerves were dissected free and their connections and distribution carefully noted. A sufficient number of specimens of each of the three species of animals were dissected to permit conclusions to be made from the results of the study. However, since Schulze and Boyden ('43) had made a detailed study of these nerves in the cat and because my dissections were made mainly on the dog, the description of the hepatic nerves will be based largely on the latter species.

RESULTS

At the outset it should be stated that this article deals only with superficial nerves lying in the lesser omentum. Specifically it omits the deeper hepatic plexus of the right gastropancreatic fold, which conveys the celiac division of the dorsal vagus to the biliary tract, and deeper branches of the ventral vagus which traverse the left gastropancreatic fold to enter the hepatic plexus. Nor does it throw any light on the existence of a coronary nerve of the lesser curvature by means of which some branches of the ventral vagus might reach the hepatic plexus.² In other words, the purpose of this article is to display those superficial hepatic branches which can be reached experimentally, in a living animal, with minimal trauma.

² The existence of such a nerve has been denied by some authors, and it may be absent in some species. By using special staining methods Johnson and Boyden have verified this pathway in the cat.

In the dog, it was found possible to trace the superficial hepatic nerves from their origin at the subdiaphragmatic vagal trunks to the choledochoduodenal juncture and the gall-bladder. In the guinea-pig it was more difficult to prevent breaking of some of the nerves, so that a dissection of an intact specimen was not secured, but it was possible to reconstruct the specimen after completion of the dissection. Since the pattern of distribution was generally similar in the two species of animals the description will be based on the dissection of the dog and supplemented, wherever necessary, by observations on the guinea-pig.

In a representative specimen (fig. 1), vagal branches to the extrahepatic biliary system (two from the ventral vagus and one from the dorsal) branch off at the level of the cardia of the stomach (the dorsal one begins at a slightly lower level than the ventral) and enter the lesser omentum, passing obliquely ventrally and to the right toward the porta hepatis. The nerves remain separate from each other before entering the latter region. One ramus from the ventral vagus gives off some small branches to the left lateral lobe of the liver. The three nerves arch superficially over the left upper edge of the papillary process of the caudate lobe of the liver. After passing between the latter and the left lateral lobe, they form a simple plexus at the cephalic edge of the porta hepatis. The main trunks, consisting of three slender nerves, continue caudally from the plexus. A small nerve accompanying a branch of the hepatic artery to the left lateral lobe of the liver runs parallel with these bundles. The two nerves on the left side unite into a single trunk which joins the bundle on the right to form the second plexus at the point slightly less than 2 cm. distal to the first plexus. It is joined by a strand from the celiac plexus. All the nerves are embedded in the adipose tissue of the hepatic pedicle as they pass caudally from the porta hepatis. They lie in a plane ventral to the hepatic blood vessels and to the left of the hepatic and common bile ducts.

A third plexus is found about 1 cm. or less distal to the second plexus. There are five strands of communication be-

tween the second and third plexuses. Anastomosing bundles from the celiac plexus, four in number, join these plexuses above and below at their medial border.

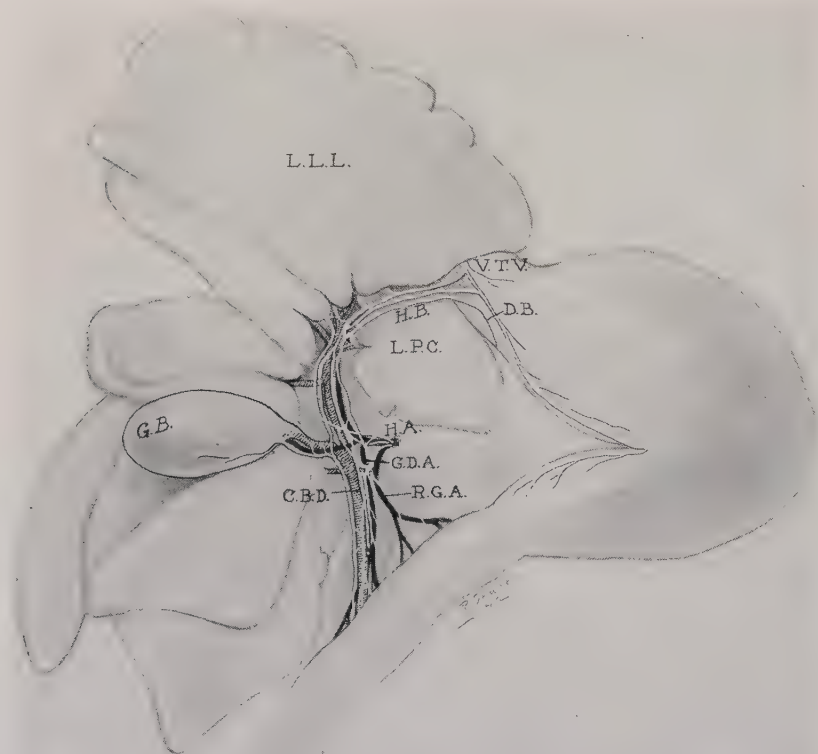


Fig. 1 Distribution of the hepatic branches of dorsal and ventral vagi of the dog: V. T. V., ventral vagus; D. B., branch from the dorsal vagus; H. B., hepatic branches of the vagi; L. L. L., left lateral lobe of the liver; L. P. C., papillary process, caudate lobe of liver; G. B., gallbladder; H. A., hepatic artery; G. D. A., gastroduodenal artery; R. G. A., right gastric artery; C. B. D., common bile duct.

A relatively large trunk is given off from the right side of the third plexus and passes between the cystic duct and the cystic artery to supply the cystic duct and the gallbladder. On the average this nerve measured 5 cm. from its origin to the neck of the gallbladder. The other nerves of the third

plexus pass caudally and spread out to form three main groups for distribution. Two strands parallel the common duct, one on each side, and then follow the ventral surface of the duct to its distal end. The second group passes along the right gastric artery to the superior pancreaticoduodenal artery and the pancreas, while the third group passes along the right gastric artery to the pylorus. A small filament passes from the plexus directly to the duodenum and at right angles to the latter.

Sections were made of the larger plexuses and these were examined microscopically to determine whether ganglion cells were present. None were found.

The pattern in the guinea-pig appeared to be much simpler than in the dog. In the guinea-pig the superficial hepatic nerves were fewer than in the dog and appeared to arise exclusively from the ventral vagus, although branches from the dorsal vagus may reach the biliary tract through the celiac division of the nerve. In this species the hepatic nerve arises from two branches which fuse into one nerve a short distance from the vagal trunk. Also there is only one plexus medial to the main hepatic duct and the nerve going toward the pylorus seems to originate from the upper hepatic plexus.

COMMENT

While simple dissections such as these do not give any idea of the complexity of the plexuses that supply the biliary tract, they do, nevertheless, point out the superficial pathways which certain branches of the vagi follow in supplying these organs. Among other things they show that in the dog the pattern of supply is especially favorable for faradic stimulation of the vagi since the superficial hepatic trunks of the vagi traverse the lesser omentum ventral to the papillary process of the caudate lobe. Furthermore in this region they can be reached before they anastomose with the branches of the sympathetic trunk coming up from the celiac plexus. To be sure they may contain ascending branches from the sympathetic system but

it is improbable that they contain efferent sympathetic nerves to the gallbladder.

By contrast most of the hepatic branches in the cat (Schulze and Boyden, '43, personal communication) lie in the left gastropancreatic fold deep to the papillary process and anastomose much sooner than in the dog with trunks from the celiac plexus, so that Johnson and Boyden ('43) in conducting experiments on this species were obliged to section the vagi at the point where the latter leave the cardiac antrum. Incidentally, these authors demonstrated that section of either dorsal or ventral vagus retards the emptying of the gallbladder, the former more than the latter since it also supplies the nerves which relax the choledochoduodenal junction.

These observations also call attention to the variations of pattern exhibited by different species of laboratory animals. In the dog, more hepatic rami arise superficially from the vagi than in the guinea-pig or the cat. In these last two species many nerves to the biliary tract traverse the deeper plexuses. Obviously, this creates difficulties of terminology. Until more species have been investigated intensively so that the features that are common to different species can be recognized, it is better not to flood the literature with new names. For this reason individual nerves have not been designated.

SUMMARY

The superficial hepatic branches of the vagi of dogs and guinea-pigs have been dissected and their course through the lesser omentum has been traced, with special reference to the nerve supply of the cystic duct and the gallbladder. It was observed that more nerves follow this superficial pathway in the dog than in the guinea-pig, so that the pattern in the former species is especially favorable for experimental procedures.

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SILVER STAINING OF NERVE AXONS IN PARAFFIN SECTIONS

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ONE PLATE (FOURTEEN FIGURES)

In the earliest techniques for axon staining the whole block of tissue was impregnated with the silver solution, exposed to the action of a reducing agent, embedded and sectioned. Later the methods were applied to frozen sections, and of recent years to mounted sections of material embedded in paraffin wax. Of these latter "silver-on-the-slide" techniques only one, that of Bodian ('36), has firmly established itself. With tissue fixed in various alcoholic fixatives it has been found to give a uniform and almost complete impregnation of all the axons in section. But in the routine use of Bodian's method on such material in this laboratory there have been found to be uncontrollable variations in the depth of staining of other elements such as Schwann cells in the sections (Holmes and Young, '42). And still more serious variability in the success of the method has been found in the study of human peripheral nerve lesions fixed in formaldehyde solutions, for with formol-fixed material the axon impregnation is often less satisfactory.

The following account is of an analysis of the factors affecting the silver impregnation of axons in paraffin sections. The empirical development of successful silver techniques has proceeded further than our knowledge of the principles involved, and the techniques are often regarded as more difficult than non-metallic staining methods, because of inexplicable variations in the results. These difficulties can only be eliminated by a further analysis of the conditions under which a successful impregnation takes place.

Thus in Bodian's method the staining agent, Protargol, is a substance of uncertain and variable composition, and no explanation has yet been advanced for the undoubted improvement given by the addition of metallic copper to the staining solution. In the course of the experiments to be described it has been found that results closely resembling those given by Bodian's method can be obtained by staining with simple buffered solutions of silver salts. The significant variables in the composition of the silver solution are its free silver ion concentration and its pH. By the use of silver nitrate solutions of different strengths, buffered at different levels of alkalinity, variations in the impregnation can be controlled. The nature of the fixative used affects not only the success of the impregnation, but also the conditions under which it takes place, and the variable success of Bodian's method with formol material is due to the use of fixing solutions of different pH. The fact that successful results are given by the use of solutions of simple silver salts demonstrates that neither the Protargol of Bodian's method nor the complex ion solutions of the classical silver techniques are essential for a successful axon stain in paraffin sections.

Bodian's method

The following is a summary of the procedure recommended in Bodian's account of his method ('36, '37).

For staining, a 1% solution of Protargol is made up in distilled water, and to each 100 ml. of this is added 5 gm. of copper. The slides are impregnated in this solution for from 12 to 24 hours at 37°C. They are then taken out, rinsed, and reduced in a solution containing hydroquinone and sodium sulphite. The slides are washed after reduction and placed in a gold chloride toning solution. The toned preparations are treated with a reducing solution of oxalic acid, and finally the residual silver salts are removed by a solution of sodium thiosulphate. The preparations, which are blue in colour, are dehydrated and covered.

Unsuccessful results with the method may arise from variations in the after treatment of the sections as well as through variations in the Protargol solution. In a previous publication details of a routine of after treatment which avoids common sources of error was described (Holmes, '42 a). This routine, although published for use after impregnation in an ammoniacal silver solution, is the one we have found best for the after-treatment of Protargol-stained material, and was based on the procedure described by Bodian.

Davenport, McArthur and Bruesch ('39) have studied the effect of using reducing solutions other than the hydroquinone-sulphite mixture in Bodian's method. They found that the completeness of the impregnation of the finest axons could be made more certain with other mixtures. However the use of different reducers is a controllable variable, and although it may in some cases improve the results, it cannot eliminate variations which have arisen in impregnation (page 175). In all the following experiments the mixture of

Hydroquinone	1 gm.
Sodium sulphite, crystalline	10 gm.
Distilled water	100 ml.

has been used.

In spite of a carefully controlled investigation of the variables in after-treatment it was found impossible to eliminate the serious variations in the success of the method with formol-fixed material. Often the nuclei and cytoplasm of fibroblasts and Schwann cells are so heavily stained that it is impossible to distinguish fine nerve axons (fig. 6), and the myelin of myelinated fibres may also take up the silver. With alcohol-fixed material the variations are never so serious but it is often inconvenient that the depth of stain of the elements other than axons is beyond control. It seemed likely that variations in the composition of the staining solution were responsible for these effects, and this possibility was examined.

All substances sold as "Protargol" are not of the same composition. Specimens manufactured by the American firm

of Bayer were found to be useless for Bodian's method, while the Protargol manufactured by the firm of the same name in Germany and by the Winthrop Chemical Company of New York were both satisfactory. Protargol is one of a class of substances often called "silver proteinates," and it is said to contain about 8.5% of silver. It is prepared by reducing silver nitrate in a solution containing the alkali degradation products of albumen but there seem to be different opinions as to the state of the silver in the resulting preparation (Sollmann, '42; Weiser, '33). Paal ('02 a, b) first used the degradation products of albumen for reducing silver nitrate, and by this means he prepared both colloidal silver and colloidal silver oxide: it seems likely that Protargol contains either or both of these compounds. The protein serves as a protective colloid for the colloidal particles in solution and also makes it possible for the substance to be kept as a dry powder which passes into the colloidal state on the addition of water.

From the specification given above a 1% solution of Protargol contains not more than 0.08 gm. of silver per 100 ml. And when the copper is added to this solution a layer of silver begins to be deposited upon it, as copper ions pass into solution and replace the silver. Consequently the addition of copper materially decreases the total silver content of the solution. Protargol solutions both before and after the addition of copper contain a very low concentration of free silver ions, and no precipitate forms on the addition of potassium chromate to the 1% solution. This observation is understandable if the silver in Protargol is in the form of metallic silver or silver oxide, for both these substances are very insoluble in water, and would not yield a sufficiently high concentration of free silver ions to give a precipitate of silver chromate.

Preliminary attempts to control Protargol staining by modifying the composition of the solution were rendered unsuccessful by the uncertainty about the nature of the substance and by the presence of the protective colloid. These experiments were therefore abandoned in favour of attempts to substitute staining solutions of known and controlled com-

position. As Protargol solutions contain a very low concentration of free silver ions inorganic silver solutions containing a low free ion content were prepared. Three alternative methods of preparing such solutions present themselves. 1. An extremely dilute solution of a soluble silver salt such as silver nitrate has a low free ion content because the total amount of silver present is small. 2. A saturated solution of a very slightly soluble silver salt has a low silver ion content for the same reason. 3. Solutions prepared by adding ammonia to silver nitrate solutions and then redissolving the precipitate in ammonia contain a low concentration of free silver ions because most of the silver in solution is bound up in the complex diamine-silver ion $\text{Ag}(\text{NH}_3)_2^+$. The following solutions were therefore tested:

1. Solutions of silver nitrate in distilled water at different concentrations, ranging between 1 part in 5000 and 1 part in 100,000.
2. A saturated solution of silver carbonate (solubility about 3 parts in 100,000), and a saturated solution of silver oxide (solubility about 1 part in 100,000).
3. Ammoniacal silver solutions prepared by adding dilute ammonia to 1% silver nitrate until the precipitate first formed was just redissolved. The resulting solution was diluted to a strength corresponding to 1 part in 10,000 of silver nitrate. In some tests an excess of ammonia beyond the amount necessary to redissolve the precipitate was added.

In these and all other experiments the mounted sections were impregnated for from 18 to 24 hours in an incubator at 37°C .: they were then treated by the published routine (Holmes, '42 a) commencing at stage 7. For reduction the hydroquinone, sodium sulphite mixture was used.

RESULTS

These solutions were first tested on sections of material fixed in:

80% ethyl alcohol	90 ml.
Glacial acetic acid	5 ml.
40% formaldehyde	5 ml.

This fixative was recommended by Bodian ('37) and in this laboratory we have found it reliable for pieces of tissue of all sizes, and the material can always be satisfactorily stained by Bodian's method. Davenport and Kline ('38) have found that mixtures of other alcohols and organic acids give slightly better results with the method, but it seems likely that the general conclusions drawn from these experiments will be applicable to material fixed in all alcoholic fixatives of this type.

The tissue was fixed for 2 days or longer, transferred to 95% and absolute alcohol, cleared in cedarwood oil and embedded. The material tested was normal dog and rabbit nerve, nerves from these animals regenerating after experimental lesions, and specimens of human peripheral nerve lesions removed at operation.

1. *Silver nitrate.* At all the tested concentrations some axon staining was given by the silver nitrate solutions, but in no case was the stain complete or satisfactory in any way.

2. *Silver oxide and silver carbonate.* The results given by these solutions were outstandingly better. A complete axon impregnation was often given, and preparations closely resembling those of the Protargol method were obtained. Unfortunately the results were not constant. Both the reagents are substances which are difficult to purify, and silver carbonate is unstable. Some of the tested specimens were prepared in the laboratory, and others obtained from commercial sources, but none could be relied on always to give a satisfactory result.

3. *Ammoniacal silver solutions.* Such solutions in which the ammonia was not present in excess often gave excellent and complete axon impregnations, but with a slight excess of ammonia the axon stain was inhibited. The results were somewhat variable, and this was attributed to the loss of ammonia by evaporation during the long impregnation.

The results given by silver oxide and carbonate and by ammoniacal silver were so markedly better, when successful, than those of silver nitrate at all strengths that it was con-

cluded that some alkalinity in the staining solution is essential for axon impregnation. For silver carbonate is slightly alkaline in solution, and silver oxide and ammoniacal silver solutions are quite strongly alkaline.

Therefore solutions of silver nitrate of various concentrations were prepared and buffered at different pH levels. The standard phosphate buffer systems could not be used as they form insoluble compounds with silver nitrate. However the buffer mixtures of solutions of borax and boric acid can be used, as the silver borates are relatively soluble, and the

TABLE 1

Volumes of 0.2 M boric acid (containing 12.4 g.p.l. H_3BO_3) and 0.05 M borax (containing 19.0 g.p.l. $Na_2B_4O_7 \cdot 10 H_2O$) mixed to give 10 ml. buffer solution at the given pH.

pH OF BUFFER MIXTURE	VOL. OF 0.2 M BORIC ACID IN ML.	VOL. OF 0.05 M BORAX IN ML.
7.4	9.0	1.0
7.6	8.5	1.5
7.8	8.0	2.0
8.0	7.0	3.0
8.2	6.5	3.5
8.4	5.5	4.5
8.7	4.0	6.0
9.0	2.0	8.0

pH range between 7.0 and 9.0 can be covered. The solutions used are 0.05 molar boric acid, and 0.2 molar borax. The following table, modified from Britton ('42) shows the volume of each solution required to make up 10 ml. of buffer at the given pH. In the experiments the buffer solutions were used both at full strength and diluted 10 times: no significant difference in the staining results was noticed. It is more convenient to use the buffer diluted 10 times, and the staining solutions were made up as in the following example. To prepare a 1 in 30,000 solution of silver nitrate at pH 7.6, 8.5 ml. of 0.2 M boric acid are added to 1.5 ml. of 0.05 M borax. The solution is made up to 100 ml. with distilled water, and 1 ml. of 0.33% silver nitrate in distilled water is added.

In order to make sure that the results obtained were not affected by the presence of the buffering ions a buffer mixture of boric acid and sodium carbonate was also tested. The results were identical with those given by the corresponding boric acid-borax solutions. However, it is quite likely that the presence of strong electrolytes in the solutions will affect the activity of the silver nitrate to some extent, as the following values for the optimal staining concentration of silver nitrate solutions are not necessarily absolute, and refer only to solutions containing also the buffering electrolytes.

The silver nitrate solutions were tested at concentrations of 1 in 10,000; 1 in 30,000; 1 in 50,000 and 1 in 100,000. Each of these was tried at various pH levels between 7·0 and 10·0, the buffers being made up according to the table, and diluted 10 times to give the final staining solution. Dilution of the buffer solution may slightly affect its pH, and the following values of the optimal pH of the staining solutions relate to the pH of the boric acid borax mixtures as given by the table, and not to their absolute pH. However colorimetric tests of the staining solutions showed that the dilution of the buffer did not affect its alkalinity by more than at the most 0·2 pH units.

It was found that quite small differences in the pH and concentration of the solution made the difference between success and failure of the axon impregnation. And similarly with staining solutions within the range which gave a good result, very small changes of pH and concentration modified the depth of impregnation of the axons and of other tissue elements such as nuclei, cell cytoplasm and collagen. And although all the tissues tested were fixed in the same mixture, and embedded in the same way, there were small variations in the composition of the solution which gave the optimal result with different specimens of tissue.

However with all the material fixed in this alcohol, formol, acetic mixture the best axon impregnation was given by silver nitrate of concentration of either 1 in 30,000 or 1 in 10,000 at a pH of either 7·8 or 8·0. Figures 1 to 4 show the effect of

variations of pH and of the concentration of the silver solution on the success of the axon impregnation. The figures are of adjacent longitudinal sections of a rabbit tibial nerve in the early stages of regeneration after an experimental section and suture. In figure 1 a 1 in 30,000 solution was used at pH 7·8. With this material it can be seen that this solution is too dilute, as the stain is feeble. Figure 2 shows a section stained in the same solution but reduced in amidol instead of hydroquinone: there is some intensification of the stain, and the fine fibers are better demonstrated. However the use of 1 in 10,000 silver nitrate at pH 8·0 (fig. 4) with hydroquinone as a reducer gives a still better result, and the preparation closely resembles those given by Bodian's method. Figure 3 represents a preparation intermediate between figures 1 and 4, being obtained by staining with 1 in 10,000 silver nitrate at pH 7·8. It is thus clear that very small variations in the composition of the staining solution have a marked effect on the result.

From these observations on the optimum pH of impregnation, it at once becomes clear why poor results were given with unbuffered silver nitrate and with ammoniacal solutions with an excess of ammonia, for the former are too acid and the latter too alkaline. As Bodian's method always gives satisfactory results with this material, though the depth of impregnation of elements other than axons is variable, it became interesting to test the pH of the Protargol solutions used in impregnation. This was done as follows.

A number of dishes containing 1% Protargol were placed in the incubator, some with copper added as in Bodian's method; others without added copper. At intervals one dish from each group was removed, and the pH of the solution tested by means of indicators in a Comparator box. The initial pH of both solutions was near to 8·2. After 2 hours the pH of the Protargol + copper solutions had fallen to 8·0, while that of the plain Protargol remained at 8·2. During the 24 hours for which the solutions were in the incubator, as in Bodian's method, the pH of the Protargol + copper solu-

tions continued to fall steadily, till at the end of the time it had reached 6.6, slightly on the acid side of neutrality. But after the same time the Protargol without copper had only fallen to 8.0.

Thus the addition of copper to Protargol solutions causes a gradual decrease in the alkalinity of the solution, and this may well be the reason for the improvement of staining that it gives. For as different specimens of material have a slightly different optimal pH for impregnation, the Protargol + copper solution will at some time during impregnation be at this optimum level, while plain Protargol will only give the best results with material which stains best at a pH between 8.0 and 8.2. The addition of copper also decreases the total silver content of the solution, but it seems likely that this effect is less important. For results equal to those of Protargol + copper impregnation are not given simply by using a more dilute plain Protargol solution.

It may be concluded that results equal to those of Bodian's method can be given by impregnation with very dilute silver nitrate in a buffered slightly alkaline solution. Small variations in the pH and concentration of the solution make a great difference to the result, and by control of these variables different specimens of tissue can be stained in a controlled manner.

Formol-fixed material

Specimens of the various types of nervous tissue mentioned above were fixed in:

40% formaldehyde		15 ml.
0.8% NaCl		85 ml.

The sections were stained with the various buffered silver nitrate solutions.

Early in the experiments it was found that the differences between different specimens of tissue which had apparently been fixed and embedded in a routine manner were much greater with formol than with alcohol material. These differences expressed themselves not only as differences in the

optimum pH and silver concentration of the staining solution, but also in the perfection of the result. Some formol material gave poorer results even with an optimum staining solution than did other specimens.

An attempt was made to analyse these differences, and it was found that the specimens differed from each other in the pH of the formol used for fixation, in the length of time they remained in the fixative before embedding, and in the rate and method of dehydration. All these variables affect the silver stain, and must be considered separately.

1. *The composition of the fixing solution.* Some histologists have stressed the necessity of using a "good" brand of formaldehyde as a neurological fixative (e.g. Russell, '39). In all these experiments the formaldehyde used was the commercial product of Messrs. Harrington of London, and no other brands have been tested.

Formaldehyde in solution undergoes some oxidation to formic acid, and consequently the solutions used in fixation may be slightly acid in reaction. Mann (1898) recommended that this acidity should be counteracted by the addition of magnesium carbonate to the solution before fixation: others have used calcium carbonate for the same purpose. There have been various opinions as to the necessity of neutralizing formaldehyde before using it as a fixative for nervous tissue. Bielschowsky ('09) recommended that it should be done, while Beech and Davenport ('33) found that the acidification of the fixative with acetic acid favored the impregnation of the finest axons by Bielschowsky's method.

I have tested the effect of fixation in formaldehyde solutions of varying pH on the effect of axon stains by Bodian's method and with the buffered silver nitrate solutions. In the first place a formaldehyde solution made up from stock 40% formaldehyde which has stood over calcium carbonate, and a similar solution containing an excess of calcium carbonate is almost exactly neutral. But a solution neutralized with magnesium carbonate is slightly but definitely alkaline: the composition of different specimens of magnesium carbonate

varies, but the formaldehyde solutions "neutralized" with the specimen used in these experiments had a pH of about 8.0. Formaldehyde may therefore be either "neutralized" (when it is made up from a stock solution standing over calcium carbonate), "neutral" (when the solution used for fixation contains an excess of calcium carbonate) or "alkaline" (when the fixing solution contains an excess of magnesium carbonate. There is an important difference between material fixed in these different solutions from the point of view of silver staining in paraffin sections. Material fixed in neutral or neutralized formaldehyde gives poor results with Bodian's method (fig. 6): the preparations are red in color, the axons are not clearly differentiated from other tissue elements, and the cytoplasm of Schwann cells is darkly stained in degenerating and regenerating nerve (this latter fact often causes serious difficulty in distinguishing between axons and Schwann cells — see Holmes and Young, '42). This material can be satisfactorily stained with buffered silver nitrate if the solution has a pH in the neighborhood of 8.4 and 8.6 and a silver nitrate concentration of 1 part in 100,000 (fig. 5).

Material fixed in formaldehyde made alkaline with magnesium carbonate, however, gives better stains by Bodian's method. The preparations are blue-black, and the axons more sharply differentiated. This material can also be stained with buffered silver nitrate, but the pH of the solution must be in the neighborhood of 7.8, and the silver concentration 1 in 10,000 or 1 in 30,000. It seems clear that the greater success of Bodian's method with the latter material is due to the fact that the optimum pH of impregnation lies within the range covered by the Protargol + copper solution, while the neutral or neutralized formol fixation requires a slightly more alkaline impregnating solution.

If no attempt is made to neutralize the natural acidity of the formaldehyde used for fixation, the optimum pH for impregnation is the same as that for material fixed in neutralized formaldehyde, and Bodian's method gives poor results.

Acidification of formaldehyde was recommended by Davenport ('34) for fixation of material to be stained by his alcoholic silver nitrate method. He pointed out that slight acidification of the fixative did much to prevent the distortion and shrinkage which formol material so often shows after paraffin embedding. I have found that tissues fixed in acid formaldehyde are much better preserved after paraffin embedding, but their reactions to the silver solutions are somewhat erratic. Sometimes good results are given by Bodian's method, and sometimes the preparations are useless. Similar variation was found with the buffered silver nitrate solutions. And acidification also causes some distortion of the axons: when 5% of acetic acid is added to the formaldehyde the axons are most abnormal in appearance, and with Bodian's stain they show a "neurofibrillar" structure which is not seen after neutral fixation.

2. *The duration of fixation.* One of the specimens used in these experiments was a human amputation neuroma which had remained in formaldehyde for 15 months before being embedded and sectioned. It was found to be remarkably easy to stain with a great variety of silver solutions. Bodian's method gave excellent results, although the formaldehyde solution used for fixation was not neutralized; ammoniacal solutions with or without an excess of silver nitrate were equally satisfactory, and complete axon impregnations were given by silver nitrate solutions over a wide range of pH and concentration. Furthermore the axon impregnation was complete even when the stage of hydroquinone reduction was omitted from the after-treatment, and the sections were transferred direct to the toning bath from the staining solution. This observation was checked by a controlled experiment with specimens of normal dog tibial nerve. Specimens of the nerve were removed at a biopsy, and fixed in neutral and neutralized formaldehyde, and in formaldehyde made acid by the addition of 5% of acetic acid. One specimen was removed from each of these three fixatives after 8 days, and the rest were left in the formaldehyde for 9 months. After this time the

long-fixed material was embedded and sectioned, and was stained with the buffered silver nitrate at the same time as the short-fixed material. As with the material described above it was found that excellent stains of the axons in the long-fixed material were given by silver solutions of a much greater variety than were successful with the other material (figs. 10 and 11). And the best preparations were superior to the best of those obtained from the short-fixed tissue.

There are two types of change which may affect the stainability of material which is allowed to remain in formaldehyde solutions. In the first place there is a gradual loss of lipid material from the myelin sheaths, as the phosphatides become converted into water-soluble substances which pass out of the tissue. In the second place there may be a progressive alteration in the properties of the proteins of the tissue.

Weil ('31), Epstein and Lorentz ('34) and others have analyzed the changes in the lipides in nervous tissue which is left for some time in formaldehyde, and they have shown that the nerve fibers are progressively demyelinated by the breakdown of the myelin sheath. The phosphatide components of the myelin become converted into water soluble substances which diffuse out of the tissue, and this liberates the galactolipides which may also migrate from their original position. One of the commonest causes of failure of a silver stain for axons is an inadequate differentiation between the axon and the myelin of myelinated fibers. With a silver solution which is not of the optimum pH or concentration the myelin may "take up as much silver" as the axon, so that the stain is non-specific (fig. 11). And with some formol material the myelin may be completely stained and the axon hardly stained at all. It might be suggested therefore that there is competition between the axon and the myelin for the silver in the staining solution, and that the axon is only specifically stained within certain rather narrow limits of staining conditions, as has been described. Thus when the fatty material from the myelin sheath had disappeared, as in long-fixed formol material, a specific axon stain can be obtained with a much

greater variety of silver solutions, as has been found to be the case.

In order to correlate the changes in the myelin sheath with the alteration of silver stainability, specimens of the tissues fixed in the experiment described, for 8 days and 9 months respectively, were stained by a modified Weigert method for myelin. At the same time as pieces of the nerve were removed for embedding, others were taken and mordanted for 14 days in 3% potassium dichromate. They were then dehydrated and embedded. Sections from the specimens fixed in acid, neutralized and neutral formaldehyde for the short period were mounted on the same slide as sections similarly mordanted after fixation for the longer period. The slides were stained with Kulschitzky's haematoxylin, and differentiated by Pal's method.

There had been a considerable loss of stainable myelin from the material fixed in neutralized formaldehyde for 9 months as compared with the control fixed for 8 days, though the myelin sheaths were still to some extent visible in the former material (figs. 7 and 8). A similar change had taken place in the material left in formaldehyde with an excess of calcium carbonate, but in the material left in the solution containing 5% of acetic acid the integrity and stainability of the myelin was quite unaffected (fig. 9). Correspondingly, in the material which had been embedded directly and stained with silver the neutral and neutralized tissues gave a specific axon stain with a greater variety of silver solutions than did the controls (figs. 10 and 11), while the silver stainability of the acid fixed material was unaffected.

It therefore seems likely that the ease with which a specific axon stain can be obtained in long-fixed material can be correlated with progressive demyelination of the myelinated fibres. The presence of acetic acid in the solution inhibits this "post-mortem change." This experiment incidentally shows that if specimens of nervous tissue are to be stored for a long period in formaldehyde the solution should be acidified if it

is desired subsequently to stain the myelin sheaths of the fibres.

The second possibility is that formol fixation affects silver staining by causing slow progressive changes in the proteins of the tissue. Spatz ('23) found that formaldehyde solutions in which tissue was stored for some months became progressively more acid, and that this acidity could not be accounted for by oxidation of formaldehyde to formic acid. He pointed out that acid substances are formed by the action of neutral formaldehyde on protein, as in Sørensen's formol titration method for the estimation of amino-acids and proteins, and he suggested that a change of this type was responsible for the development of acidity in the tissue and the fixing fluid, and for observed changes in the stainability of long-fixed tissues. Zeiger ('30) showed that formol-fixed protein is more acid than alcohol-fixed, and that this electrostatic difference has an important effect on the staining reaction of the tissues to solutions of different pH.

However it seems likely that the development of acidity in formol-fixed tissues and in the solution containing them is caused by the hydrolysis of the phosphatides produced by the breakdown of the myelin rather than to progressive changes in the protein. But it cannot be excluded that some change does take place in formol-fixed protein allowed to remain for a long time in the fixative. Formaldehyde acts as a fixative by rendering the colloidal proteins resistant to precipitation by alcohol: the chemistry of this reaction has been extensively studied as the action of formaldehyde on the protein casein is one of the methods by which "plastics" are prepared industrially. The reaction takes place slowly — 24 hours action is necessary for the "fixation" of a sheet of casein 2 mm. thick (Morrell, '37) — and the substance formed retains to some extent the colloidal property of swelling when soaked in water: a rod of formol-casein plastic will increase in diameter by 10% during 21 days soaking. Underhill ('32) has verified histologically the slow rate of action of formaldehyde on

proteins, but at present we have no information on the behavior of the fixed protein when stored in the fixative.

If a transverse section of a normal peripheral nerve fixed in an alcoholic fixative is compared with one fixed in formol it can be seen that the axons of the large fibers are considerably more shrunk in diameter in the former than in the latter. And in silver preparations of alcohol-fixed material the axons are always much more darkly stained than in formol material, and consequently are better differentiated from the background. It therefore seems likely that a better specific axon impregnation is given in material in which the axons have undergone shrinkage by protein precipitation and dehydration. Although formaldehyde protects proteins from precipitation by alcohol, some shrinkage takes place during dehydration and embedding of formol material, and as with all tissues the amount of shrinkage depends on the mode of dehydration, the temperature of the paraffin oven and other factors. Consequently it may be expected that different specimens of formol material will give varying successful axon impregnations according to the amount of shrinkage the axons have undergone. It has been pointed out that better axon stains are often given when formol material is transferred directly from the fixative to 95% alcohol in dehydration (Holmes, '42 a), and I have noticed that in sections of sympathetic ganglia the nerve cells which are obviously shrunken stain more darkly with silver than those which are not.

"Post-fixation" of formol material

Several procedures have been introduced into neurological technique in which formol-fixed blocks of tissue or frozen sections are treated with some special reagent before impregnation.

Bielschowsky ('09) treated material for 1 to 4 days with pure pyridine before impregnation, and he considered that the reagent acted as a fat solvent, making staining easier by demyelinating the fibers. Landau ('24) produced evidence that fat extraction does improve staining: he treated blocks

of tissue with a mixture of alcohol and ether in a Soxhlet apparatus for 24 hours, and found an improvement in the axon stain. The experiment described on page 171 also gives evidence that demyelination aids axon impregnation, but Landau's experiment does not prove that pyridine acts as a fat solvent in Bielschowsky's method. Formaldehyde does not fix fats, so treatment of paraffin sections with pyridine should produce a similar improvement of the stain. I have treated $10\ \mu$ paraffin sections for 24 hours with pure pyridine at 37° , but find that even after this procedure a silver solution which is not of the optimum qualities for the demonstration of axons stains the myelin as completely as in control material. Similarly in the silver method already published (Holmes, '42 a) a preliminary treatment of the sections for 48 hours in a warm mixture of xylol and glacial acetic acid was recommended. This gave a considerable improvement in the sharpness of the axon stain with the ammoniacal silver solution used, and it was thought to achieve this by demyelinating the fibers. However further work has shown that the myelin is not in fact attacked by the xylol acetic mixture, and the improvement of the stain must be due to some other effect.

It has been known for some time that the electrostatic condition of fixed tissues has an important effect on their staining reactions. Silver ('42) has given a clear demonstration that this is true in silver staining, and has also shown that the nature of the charges on the tissue elements can be modified by treatment of the sections with various reagents. In the case of the xylol acetic treatment there seems no doubt that the beneficial effect is the result of some such change in the tissue, and it is also likely that the same is true of other methods of pretreatment. Treatment of sections with permanganate and oxalic acid, for example, in the "Mallory bleach" which is an essential preliminary to the silver impregnation of reticulin fibrils, transfers the specific stain away from the axons. Pyridine has been used in axon staining in aqueous solution, as in Agduhr's method ('30), so cannot be functioning as a fat solvent. In Stöhr's modification of Schultze's

method the frozen sections are treated before impregnation with caustic soda of varying concentration, the strength of solution depending on the nature of the tissue. If the alkali were functioning as a demyelinating agent by saponifying the fats of the myelin, it is difficult to see why its concentration should be critical. And as the lipides in the myelin sheath may in part be bound to protein molecules it is unlikely that alkali treatment would remove a significant proportion of the myelin. We have seen that the difference between alkaline and neutral formaldehyde as a fixative makes a considerable difference to the axon stain, so it seems possible that the role of alkali treatment and of treatment with pyridine, which is also an alkali, is primarily to modify the electrostatic condition of the tissues.

The reducing solution

The properties of the reducing solution into which the sections are placed after silver impregnation have often been considered to have a critical effect on the success of the axon impregnation. Kubie ('29), discussing methods for frozen sections, considered that when the section is removed from the silver solution and placed in the reducer the silver solution with which it is "soaked" begins to diffuse out, so that if the rate of reduction is not properly adjusted to this rate of diffusion the metallic silver liberated by the reduction will be deposited on the surface of the section, instead of within it on the axons. Davenport, McArthur and Bruesch ('39) in their study of Bodian's method have stressed the importance of the pH of the reducing solution. Silver ('42) has carried this idea further. He considers that the site of deposition of the silver i.e. the specificity of the stain for the axons, is determined very largely by the pH of the reducing solution; the charged ions in the solution modify the charges on the tissue elements, and thus have a most critical effect.

Experiments with dilute silver solutions show that the factor of rate of action of the reducer and rate of diffusion of the silver solution out of the tissue is not significant in

the staining methods for paraffin sections. The slides can be transferred to distilled water from the silver bath and left there for a sufficient time for all the contained silver solution to diffuse out without impairing the specificity of the stain. I have also tested the effect of using silver solutions of different pH. Hydroquinone is only active in alkaline solution: the hydroquinone-sulphite mixture has a pH of about 10. However, amidol is a photographic reducer which is active at neutrality as well as in alkaline solution, and by its use in solutions containing various mixtures of sodium sulphite and sodium bisulphite it can be tested at different pH levels. I find that the pH of the reducing solution does not of itself affect the specificity of the stain. The only differences between the preparations can be attributed to the fact that hydroquinone is less active in less alkaline solutions and that at all levels of alkalinity amidol is a stronger reducing agent than hydroquinone. The effect of using a stronger reducer or a more alkaline solution is only to make the stain of all the tissue elements including the axons more intense. It probably also serves to bring out more of the fine non-medullated axons. It was for this last purpose that Davenport and his collaborators suggested the use of a mixture

Amidol	0.5 gm.
Sodium sulphite crystals	10 gm.
Sodium bisulphite	2.5 gm.
Water	100 ml.

This reducer is less alkaline than the hydroquinone sulphite mixture, but gives a darker stain because of the greater activity of amidol as a reducing agent. The use of such a solution may be valuable not only for bringing out fine axons, but also when required for improving the impregnation of other structures such as Schwann cells after staining with buffered silver nitrate.

It may be concluded that the pH of the reducing solution is not critical in determining the specificity of the stain either in Bodian's method or after the use of buffered silver nitrate.

Picric acid fixation

In earlier work on crustacean nerve fibers (Holmes, '42 b) it was found that excellent axon impregnation was given by the use of Bodian's method on material fixed in a saturated solution of picric acid in isotonic saline. In the present series of experiments specimens of human and mammalian peripheral nerve were fixed in a saturated solution of picric acid in 0.7% saline and others in a similar solution with the addition of 5% of acetic acid. Picric acid penetrates relatively slowly (Medawar, '41) so the addition of the much more rapidly penetrating acetic acid seemed desirable in order to prevent post mortem changes in large blocks of tissue fixed by immersion. After fixation for a sufficient time to allow complete penetration by the picric acid the material was embedded and sectioned. Staining with the buffered silver nitrate solutions gave an excellent axon impregnation both with 1 in 10,000 silver nitrate at pH 7.8 and a 1 in 100,000 solution at pH 8.4. The axons were sharply differentiated, and the impregnation was as complete as in the most successful stains of alcohol fixed material (fig. 14).

It seems significant that while there was no observable difference between the impregnation of the tissues fixed in the simple picric solution and that of the picric-acetic material, results given with Bouin's fluid, which is the picric-acetic solution with added formaldehyde, were markedly less satisfactory and more variable. It may be concluded that an optimal axon impregnation is given after fixation in picric acid because it is a protein precipitant: acetic acid is without effect on proteins, so does not modify the impregnation. Formaldehyde however does do so, for it penetrates more rapidly than picric acid and protects the proteins from precipitation.

The choice of a fixative for peripheral nerve

In animal experiments sufficient material is usually available for different specimens of the peripheral nerves to be fixed in different fixatives each chosen to give an optimal result

with a different staining method. But in the study of human peripheral nerve lesions it is necessary to obtain the maximum amount of information from each individual specimen, and it is seldom convenient to use a variety of fixatives in the operating theatre. Formol fixation is excellent for myelin sheath staining in paraffin sections if the fixed tissue is mordanted in the usual way before embedding. But for other purposes formaldehyde has great disadvantages. Axon impregnation is possible, as has been described, but the variations in the result are inconvenient and may be misleading. And a still more serious objection is the fact that peripheral nerve undergoes very serious distortion during paraffin embedding after formol fixation. Figures 12 and 13 are transverse sections of a small branch of a human lateral popliteal nerve. The main trunk of the nerve had been interrupted by a gunshot lesion some time previously, so the branch was completely degenerate. The nerve was removed at operation, and divided into two pieces, one of which was fixed in formol-saline, the other in picric acid-saline. The pieces were carried through dehydration, clearing and embedding together. Figure 12 shows the appearance of the section of the picric fixed material, figure 13 that of the formol-fixed material. So great is the distortion of the latter that it seems almost incredible that the two sections are at levels separated only by a few millimeters.

Alcoholic fixatives give excellent axon impregnations with Bodian's method and with buffered silver nitrate, but it seems likely that still better results would be given by omitting formaldehyde from such mixtures, in order to allow the alcohol to exert its full effect as a protein precipitant. Fixation in the picric-acetic mixture however has the advantage that the myelin sheaths are less distorted than by alcohol and can be demonstrated, though not as clearly as in formol material, if the tissue is chrome-mordanted after fixation.

Peripheral nerve lesions fixed in formol or alcohol sometimes give trouble in section cutting because of the hardness of the masses of fibrous tissue that they often contain. Picric

acid however does not harden the tissue so strongly, and section cutting is much easier. For the same reason picric acid might be useful for the fixation of blocks of muscle for paraffin embedding and axon staining.

DISCUSSION

This work has given evidence that the success of Bodian's method for staining nerve axons in paraffin sections is due to the low free silver ion content and controlled hydrion concentration of the staining solution. Consequently it is possible to substitute a simple inorganic silver solution for the Protargol + copper solution with equally successful results. The use of very dilute silver nitrate in a buffer solution enables the specificity and completeness of the impregnation to be completely controlled, and the staining solution modified so as to be of an optimal pH and concentration for tissues fixed in different fixatives. The optimum pH for axon impregnation varies according to the nature of the fixative used, and as silver salts are more easily reduced in more alkaline solutions, a more dilute silver solution should be used for the staining of tissues which require a more alkaline solution for an optimal impregnation.

In Bielschowsky's method for the impregnation of axons in frozen sections the sections are first treated for many hours in a strong solution of silver nitrate, and then placed for a short time in an ammoniacal silver solution. This is prepared by adding caustic soda to a solution of silver nitrate and dissolving the precipitate formed in ammonia. Rio-Hortega ('17) used a similar procedure, but prepared the ammoniacal solution by adding sodium or lithium carbonate to the silver nitrate and dissolving the precipitate of mixed silver carbonate and hydroxide in ammonia. Kubie and Davidson ('28) have discussed the composition of these ammoniacal silver solutions, and point out that in all of them the silver is present in the form of the diammine silver complex ion, and that they differ from each other only in their other components. These differences affect the pH of the solution, and Kubie and

Davidson suggest that the different uses of ammoniacal solutions may be due to these variations in the degree of alkalinity.

When attempts were first made to stain axons in paraffin sections the ammoniacal silver solutions were used. However the high total silver content of the solutions often caused a deposition of metallic silver on the surface of the sections, and their high alkalinity tended to detach the mounted sections. These difficulties were to some extent overcome by a variety of modifications, but the first significant step towards avoiding them was made by Bartelmez and Hoerr ('33). They modified an earlier method of Rogers ('31) by replacing the ammoniacal impregnating solution by a dilute solution of Protargol. That is to say that they replaced a strongly alkaline solution of high silver content by a slightly alkaline solution of low silver content. Then in 1936 Bodian found that the addition of metallic copper to the Protargol solution was followed by an improved axon stain.

The differences between successful methods for frozen sections and successful methods for paraffin sections are therefore as follows. For frozen section staining the sections must be treated for many hours in a strong solution of plain silver nitrate: I have found that such a procedure does not improve impregnation in paraffin sections either in Bodian's method or in staining with buffered silver nitrate. The sections are then transferred for some minutes only to a strongly alkaline solution containing a high concentration of the diammine silver ion, and then reduced. In the methods for paraffin sections a long impregnation is necessary in a slightly alkaline solution of a low total silver content.

When paraffin sections are taken out of either Protargol or buffered silver nitrate they are pale brown in color, and when placed in the reducing solution they immediately become much darker brown by a further reduction of silver ions to metallic silver. If a frozen section is placed in a buffered silver nitrate solution of a similar composition at 37° it becomes progressively darker brown during impregnation and does not change in color at all when placed in the reducer.

That is to say that all the silver solution which penetrates the section is immediately reduced by the tissue, and none remains to be reduced by the reducing solution. I have tested dilute buffered silver nitrate solutions at all pH levels between 7.6 and 12.0 and find that in no case can a successful axon stain be obtained by following the same procedure as is successful with paraffin sections.

It may thus be suggested that frozen sections differ from paraffin sections in that the fixed but unembedded tissues are much more powerful in reducing silver solutions, however slightly alkaline they are. This reducing power is general throughout the tissue, so no specific stain results. A preliminary treatment with strong silver nitrate at room temperature for many hours "exhausts" much of this reducing power, and the tissue becomes pale brown in color. The frozen section is then transferred to an ammoniacal silver solution which is at the optimum pH for the impregnation of the axons. The tissue is left for a short time in this solution, and on being transferred to the reducer the ammoniacal silver ions with which it is permeated are converted to metallic silver particles, and these are deposited around the silver "nuclei" formed on the axons during the ammoniacal silver treatment. A dilute buffered silver nitrate solution cannot be substituted for the ammoniacal solution in this procedure because it has such a low total silver content that an inadequate amount of silver is liberated when the section is transferred to the reducing agent.

The significant qualities of ammoniacal silver solutions may be summarized: (1) They are at the optimum pH for the formation of nuclei of silver on nerve axons in frozen sections whose "general reducing power" has been exhausted by preliminary treatment with silver nitrate. This pH is governed, as Kubie and Davidson showed, by the nature of the reagent used to precipitate the silver before its resolution in ammonia. (2) They must contain a definite but low concentration of free silver ions in order to effect this nucleation before reduction. A diammine silver solution contains a very low

concentration of free silver ions, and these are furnished by a secondary ionization of the diammine complex ion. This secondary ionization yields both free ammonia and free silver ions, so consequently it is repressed by the presence of an excess of ammonia. Hence the importance which is always attached to not adding an excess of ammonia to silver solutions, or in adding a definite small excess, lies in the effect of the excess on the concentration of free silver ions. (3) The solutions must contain a high total silver content which is available for reduction. The use of complex silver ions is the only means by which an alkaline solution with a high total silver content can be prepared. For if the silver is in the form of a highly ionized substance the very insoluble silver hydroxide will be precipitated from the alkaline solution.

Thus it may be concluded that the difference between successful methods of axon staining in frozen and paraffin sections arise from changes during dehydration and embedding in the effect of the tissue on silver solutions.

In this discussion we have adopted Liesegang's view ('11) that silver staining involves two stages in the reduction of silver. First, in the staining bath some silver is reduced and deposited in the tissue as nuclei. The distribution and characteristics of these nuclei determine the specificity of the stain. Then when the section is transferred to the reducer more metallic silver particles are liberated, and are deposited in relation to these nuclei.

Silver ('42) has presented most valuable evidence for the theory that the specific deposition of silver in staining is brought about by the localized precipitation of charged silver micelles by the oppositely charged tissue elements. He considers, however, that the specific deposition takes place at the moment at which the section is placed in the reducing agent, and would thus seem to reject Liesegang's view. But the following experiment is ample evidence that the specificity of the stain is determined by nucleation during impregnation.

Two sets of mounted sections of nervous tissue were impregnated with buffered silver nitrate. One set was immediately

carried through the usual routine of reduction, gold toning and oxalic acid reduction. Those of the second set were washed in distilled water for 6 hours after impregnation and before reduction. When they were eventually placed in the hydroquinone reducer after this long wash they showed little or no darkening of their brown color, indicating that most of the silver available for reduction had been washed out. They were then toned for 3 minutes and placed in the oxalic acid. Here the nerve axons rapidly became visible, and after dehydration and covering the axon stain was found to be as complete as that in the control series. The only possible interpretation of this experiment is that specific nucleation took place in the 24-hour staining with silver nitrate. After washing no more silver was available for reduction, but during toning the silver nuclei were replaced by gold nuclei, and the section was soaked in gold chloride. Subsequent reduction caused deposition of metallic gold around the nuclei, and a complete stain resulted. That is to say that the conditions of pH in the first reducing solution do not affect the specificity of the stain, which is determined during impregnation.

SUMMARY

1. Nerve axons in mounted paraffin sections can be satisfactorily stained with silver nitrate, providing that the solution is very dilute and buffered to slight alkalinity.
2. Material fixed in an alcohol-formol-acetic fixative can be stained with 1 in 10,000 silver nitrate at pH from 7.8 to 8.0.
3. Material fixed in unneutralized or neutralized formaldehyde can be stained with 1 in 100,000 silver nitrate at pH 8.4.
4. Material fixed in formaldehyde made slightly alkaline with magnesium carbonate can be stained by 1 in 10,000 silver nitrate at pH 7.8 to 8.0.
5. Material fixed in a solution of picric acid with or without acetic acid can be stained with either 1 in 10,000 silver nitrate at pH 7.8 or 1 in 100,000 silver nitrate at pH 8.4.

6. By variation in the concentration of the silver solution and in its pH the stain can be controlled and adapted to different tissues and different purposes.

7. It is suggested that Bodian's method owes its success to the fact that the solution of Protargol with added copper contains a very low concentration of silver ions and has a pH which slowly falls from about 8.2 to below neutrality.

8. The variable results of Bodian's method with formol-fixed material are due to the fact that the Protargol solution never reaches the alkalinity which is necessary for an optimal impregnation of material fixed in the unneutralized fixative.

9. Several variables in fixation and embedding which affect the success of axon stains are discussed.

10. The specificity of the stain is determined during impregnation by the effect of the pH and concentration of the solution on the formation of nuclei of reduced silver in the section. At subsequent reduction more silver is deposited in relation to these nuclei, but the conditions in the reducing solution do not affect the specificity of the stain.

11. Methods for axon staining in frozen sections are not different in principle from those for paraffin sections, but different procedures are necessary because the tissue reacts differently with silver solutions when it has not been dehydrated and embedded.

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PLATE 1

EXPLANATION OF FIGURES

Figs. 1 to 4 Adjacent longitudinal sections of the tibial nerve of the rabbit, 15 days after section and immediate suture. The figures show a point about 5 mm. distal to the site of suture, and represent the degenerating nerve in the process of reinnervation. Alcohol, formol, acetic fixation. All to the same scale.

1 Stained with 1 in 30,000 silver nitrate at pH 7.8: hydroquinone reduction. The sections are pale bluish-green in color, the nuclei are hardly stained, and the axons are not sharply and completely demonstrated.

2 Stained with the same solution as the section in figure 1, but reduced with amidol. The stronger reducer has brought out the nuclei and axons better.

3 Stained with 1 in 10,000 silver nitrate at pH 7.8: hydroquinone reduction. The increase in concentration of the silver solution over that used in figure 1 has improved the quality of the stain, but it is still rather faint.

4 Stained with 1 in 10,000 silver nitrate at pH 8.0. The slight increase in pH over that used for the section in figure 3 has greatly improved the quality of the stain, which resembles preparations made by Bodian's method.

5 Longitudinal section of a human peripheral nerve at a point distal to a gunshot lesion some months previously. The nerve is degenerate and contains proliferated Schwann nuclei with their cytoplasm, but one regenerating nerve fiber that has succeeded in crossing the lesion can be seen. Fixation in unneutralized formol saline: stained with 1 in 100,000 silver nitrate at pH 8.4.

6 An adjacent section of the same material as that in figure 5: stained by Bodian's method. The stain is very dark, and the fibrous cytoplasm of the Schwann cells has taken up the silver. Consequently it is almost impossible to distinguish nerve axons from Schwann cell cytoplasm. Same scale as figure 5.

Figs. 7 to 9 Transverse sections of normal dog sciatic nerve, the material being mordanted after fixation with potassium dichromate, and stained with Kulschitzky's haematoxylin to demonstrate the myelin sheaths. All to the same scale.

7 Specimen fixed for 8 days in neutralized formaldehyde before mordanting.

8 Fixed for 9 months in neutralized formaldehyde before mordanting.

9 Fixed for 9 months in formol containing 5% acetic acid.

Figs. 10 and 11 Longitudinal sections of normal dog sciatic nerve mounted and stained on the same slide with 1 in 10,000 silver nitrate with just enough ammonia added to redissolve the precipitate.

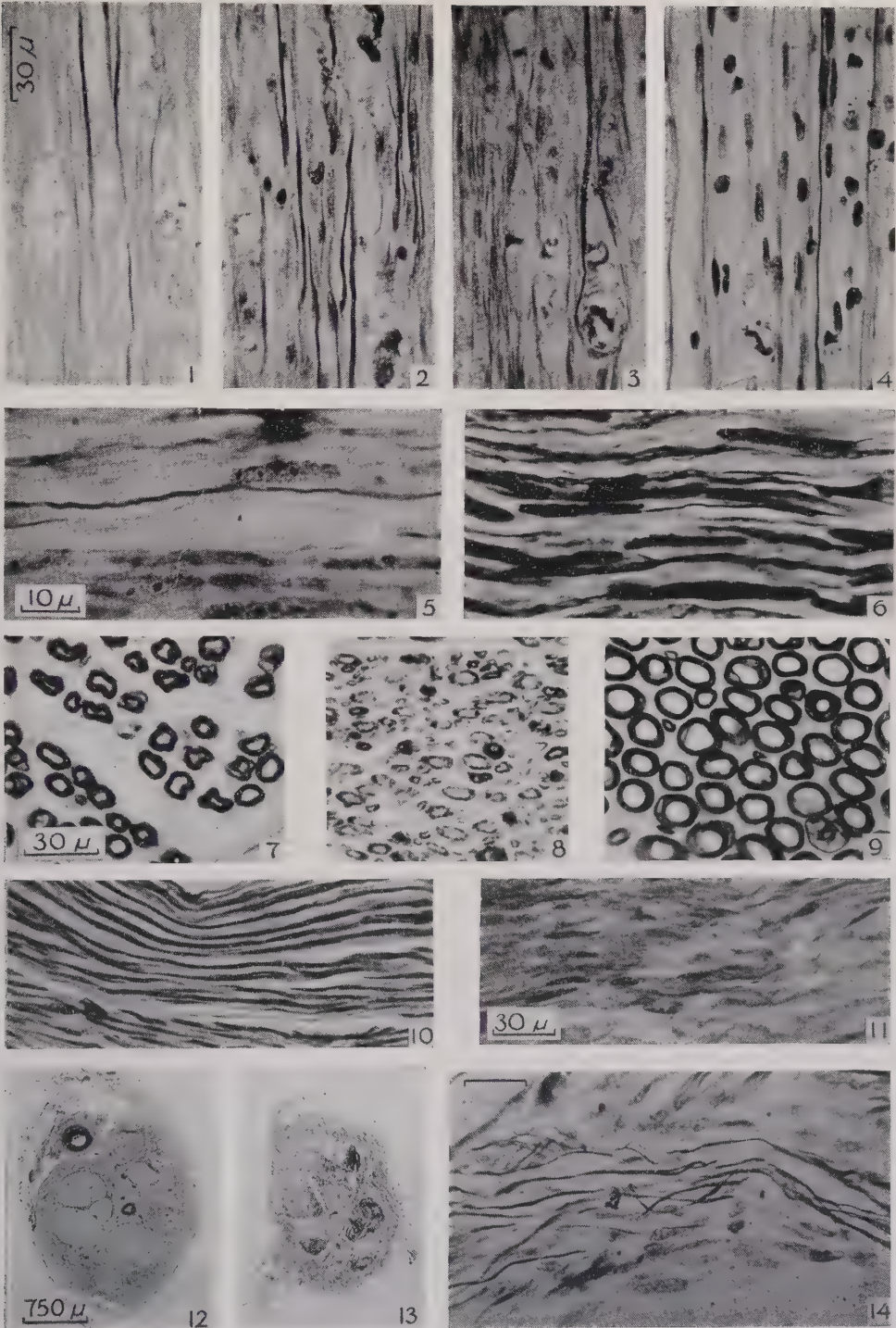
10 Material fixed for 9 months in formol-saline.

11 Material fixed for 8 days in formol-saline: only the nuclei in the section are specifically stained; the axons, myelin and connective tissue have all taken up the silver to the same extent.

12 Human peripheral nerve fixed in a saturated solution of picric acid in 0.7% saline. Van Gieson (see text).

13 Same nerve as in figure 12 fixed in formol-saline. Van Gieson (see text).

14 Nerve axons in the neuroma formed after division of a human ulnar nerve by a gunshot lesion. Fixed in a saturated solution of picric acid in 0.7% saline: stained with 1 in 100,000 silver nitrate at pH 8.4.



A TECHNIQUE FOR THE GROSS DIFFERENTIAL STAINING OF PERIPHERAL NERVES IN CLEARED VERTEBRATE TISSUE

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ONE PLATE (TWO FIGURES)

This technique had its inception several years ago when we were searching for methods of demonstrating nerve and bone tissue in cleared, whole vertebrate forms. In the course of our investigations we encountered a French work by Pollack ('00). Among others of the more common methods for staining nerve tissue discussed in this book was one, formulated by a Charles Sihler, which particularly aroused the curiosity of the present writer because of the possibilities of modifying and adapting it to gross transparent preparations. A search of literature failed to reveal that Sihler had ever published this technique. More recently our attention was called to a paper by Wharton ('37) who also employs Sihler's method, with modifications, for staining nerve tissue in gross preparations. The only reference Wharton was able to find to Sihler's method was in a work by Mann ('02); even here there is a possibility, as Wharton points out, that the Sihler referred to is not the same individual who formulated the method under discussion. Wharton (*loc. cit.*) obtained the essentials of the procedure from formulae on index cards belonging to Mr. Charles Miller, of the Department of Embryology, Carnegie Institute; these were accredited to Dr. Charles Sihler, many years ago associated with the Department of Biology, Johns Hopkins University.

In preparations made by the Sihler method, as described by Pollack (*loc. cit.*), nerves innervating various organs are made

visible only after small pieces of the tissue have been treated and then teased completely apart; even then the non-nervous tissue remains heavily stained, with consequent loss of differentiation. Myelinated fibers are stained slightly heavier than the surrounding tissue but there is little or no differentiation of non-myelinated fibers.

As the present technique differs to some extent both in procedure and results from that described by Wharton (*loc. cit.*), we feel justified in publishing this method. The major differences between the two techniques involve methods of fixation, maceration in KOH to facilitate later destaining, depigmentation, dehydration and clearing; procedures which were first devised by the author ('41) in a technique for differentially staining bone and cartilage in cleared, gross vertebrate specimens.

TECHNIQUE

1. Fixation and hardening

The necessity for fixing the tissue as soon as possible after death cannot be over-emphasized as an important factor in this technique. Post-mortem changes occur rapidly in nerve tissue, unless it is fixed immediately, and frequently cause defective staining. As myelin is lipoidal in character, a 10% solution of commercial formalin is recommended as the best fixing reagent for this method; the slight amount of formic acid in commercial formalin is less harmful in its effects upon nerve tissue than a neutralized solution. We also have determined that formalin is the safest reagent to use in hardening tissue which is macerated subsequently in KOH. To insure quick penetration in larger forms, the solution is injected into the body cavities; or, one may make a mid-ventral incision along the abdominal wall. Complete evisceration is recommended also when convenient. The tissue should remain immersed in several changes of the formalin solution for at least 1 week before maceration.

2. Maceration and depigmentation

Macerating the hardened tissue in a 3% aqueous solution of KOH serves several purposes: it facilitates penetration of subsequent solutions and makes possible the maximum destaining of non-nervous tissues. At the same time it dispenses with the necessity of using the troublesome methods of bleaching heavily pigmented tissues with hydrogen peroxide or chlorine. The period of time for which the specimen should remain in the KOH solution depends primarily upon its thickness; 18 days are usually sufficient providing the tissue has been well hardened. The process is hastened if it is allowed to take place under ultra-violet rays. The KOH solution should be changed every 2 days, especially in the case of heavily pigmented forms. Frequent rotation of the specimen is suggested also. At the end of maceration the tissue should appear well bleached and translucent; superficial bony structures should be visible beneath the integument.

3. Staining

The specimen is immersed for 2 days in the first of Sihler's two solutions, made as follows:

Glacial acetic acid	1 part
Pure glycerin	1 part
1% aqueous solution of chloral hydrate	6 parts

The chloral hydrate in this solution is not absolutely essential when the tissue has been macerated previously in KOH. Its purpose, as stated by Gatenby et al. ('37) is to revive the susceptibilities of the tissues to further impregnation. The solution should be made up at least a week in advance of its use, in order to prevent the formation of bubbles in the tissue. If bubbles should form they may be eliminated by placing the tissue and solution in a vacuum chamber.

The tissue is next transferred to Sihler's staining solution for at least 4 days (2 days are sufficient for small specimen) to insure thorough penetration:

Ehrlich's hematoxylin	1 part
Pure glycerin	1 part
1% aqueous solution of chloral hydrate	6 parts

The staining solution may be used several times.

4. *Destaining*

Destaining is carried out in the acetic acid-glycerin-chloral hydrate solution mentioned above. The tissue may be left in this solution, without particular attention, for 2 hours; after that it is best to observe the process rather carefully under the low power of a binocular dissecting microscope, using strong transmitted light. The tissue should be agitated frequently and the solution should be changed when it becomes too discolored. To know exactly when to stop destaining is a matter gained by experience; however, when the stain begins to leave the finer nerve twigs the process has gone far enough. At this point in the procedure, before clearing, non-nervous tissue such as muscle may not appear completely destained. Later treatment will help to clear it considerably more.

5. *Dehydration and clearing*

Dehydration is accomplished by running the specimen through two changes of cellosolve of 6 hours each. For small pieces of tissue this time may be considerably reduced. Cellosolve, in addition to dehydrating the tissue, also renders it slightly alkaline, turning the stained nerves a deep blue and other tissues a contrasting light pink. Though not as effective, the alcohol series may be substituted for the cellosolve: 50%, 80% and 90%, followed by three changes of benzene. If the alcohol is used the destained tissue must first be made alkaline by washing it in a weak aqueous solution of lithium carbonate.

We (loc. cit.) have found that the best medium for clearing the specimen after dehydration is methyl salicylate (synthetic oil of wintergreen), which possesses essentially the same index of refraction as the tissue to be cleared. From the cellosolve (or benzene) the specimen is transferred to solu-

tions of 25%, 50% and 75% of methyl salicylate in cellosolve, for 6 hours each. It is then placed in pure methyl salicylate where it will continue to clear for some time.

6. Storage and mounting

The specimen may be stored permanently in methyl salicylate. Tissue so kept may turn slightly brown over a period of years but this may be remedied by transferring it to a fresh lot of the oil and re-clearing. Alternatively, one may mount small, whole specimens, or parts of specimens, in Canada balsam on slides; for this type of mount we ('43) have devised a method for making micrological deep-cells of laminated celluloid, which will accommodate large, irregularly shaped preparations. Photographs of two of our nerve preparations, mounted on slides in Canada balsam, are included in this paper.

If, at the end of the procedure, the tissue should appear over-stained, it may be destained as follows: place the tissue back into two changes of cellosolve of 6 hours each; then into Sihler's first solution until sufficiently destained, when it may be run up through the cellosolve and methyl salicylate mixtures as outlined above.

REMARKS AND DISCUSSION

By means of this method we have obtained well stained preparations of the peripheral and, in many instances, the central nervous systems of forms representing nearly all the vertebrate classes. The technique, applied to mammalian tissue, is uncertain and it is with this group that we have had the least success. We have succeeded in staining the nerves of a human foetus of about $3\frac{1}{2}$ months development, but the nerves are not stained deeply and are therefore somewhat difficult to differentiate from surrounding tissues. The cause of this difference in the specificity of the stain for mammalian tissue has not been determined. In all instances the stain is more specific for myelinated than for non-myelinated nerves. In the majority of whole preparations made, especially those of the smaller fishes, amphibia and reptiles, the non-nervous

tissues, even bone, appear well cleared and stained a light pink; the nerves are stained blue in strong contrast. The transparency of the preparations permits an exceptionally deep field of observation, so that the relations of the nerves to surrounding structures are readily seen; in this respect plexuses and the origin of spinal nerves are particularly striking.

Tissue prepared by this method can be handled with facility while in the clearing oil. In instances where transparency is impaired, due to thickness of the tissue, one may resort to gross dissection in obtaining blocks of convenient dimensions. Fine dissections or detailed observations of the tissue may be carried out readily under the low, medium or high powers of a binocular dissecting microscope, using transmitted light for the purpose.

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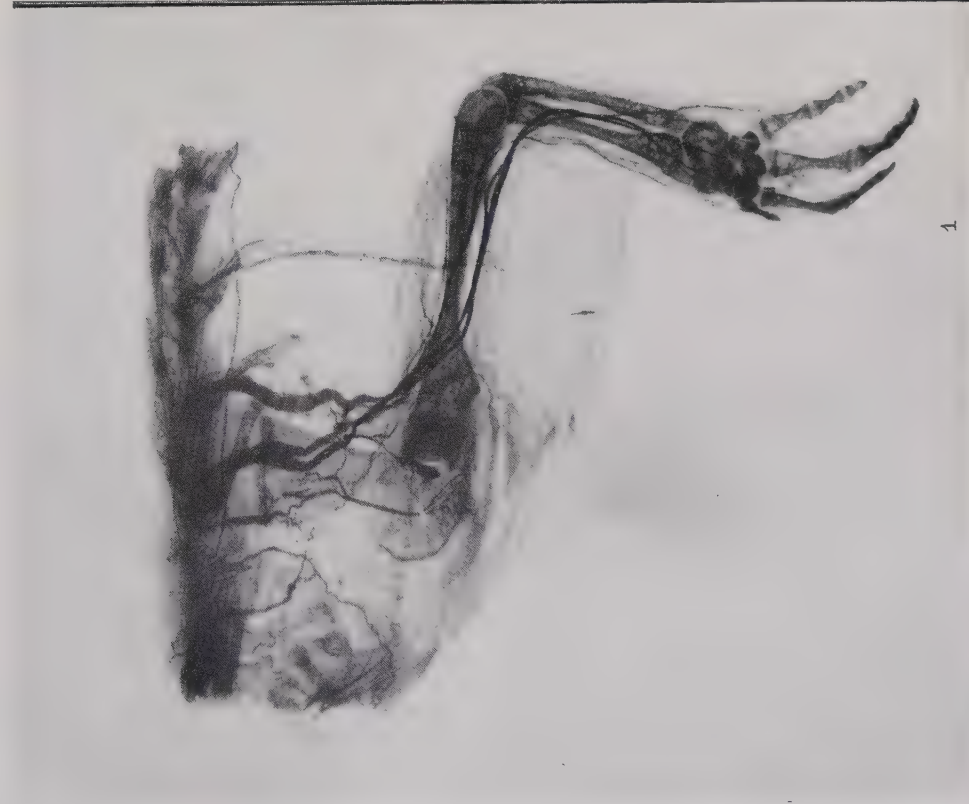
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PLATE 1

EXPLANATION OF FIGURES

1 Photograph of the left thoracic region, arm and hand of the amphibian, *Triturus viridescens* v., $\times 7\frac{1}{2}$. Lateral view. Soft tissues cleared, nerves stained. The preparation demonstrates the relation of the brachial plexus of nerves to the pectoral girdle and their distribution to the musculature and integument of the body wall, arm and hand. The origin and distribution of the dorsal rami may also be seen. (Balsam mount.)

2 Photograph of the dorsal aspect of the right leg and foot of the amphibian, *Triturus viridescens* v., $\times 11$. Soft tissues cleared, nerves stained. The preparation demonstrates the distribution of nerves to the leg musculature and integument and to the dorsal and ventral aspects of the foot. (Balsam mount.)



THE ATTAINMENT OF SEXUAL MATURITY IN THE FEMALE ALBINO RAT AS DETERMINED BY THE COPULATORY RESPONSE ¹

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ONE TEXT FIGURE AND ONE PLATE (SIX FIGURES)

In the laboratory rat, many details of the first reproductive cycle associated with the onset of puberty are either incomplete or totally lacking. The marked discrepancies in the reported age at which the female albino rat becomes sexually mature have been due primarily to the variations and limitations of the criteria used to determine the onset of the first reproductive cycle. Vaginal introitus, changes in the vaginal smear, sexual receptivity and ovulation are all integral factors in the attainment of sexual maturity. Since it is conceivable that there could be considerable variation in the time at which each of these factors becomes established, it would seem important to correlate at least several of these phenomena in order to define the onset and attainment of sexual maturity.

To date, the most complete study of sexual maturity in the female rat is that of Long and Evans ('22). By investigating the vaginal smear and the presence of the copulation plug, as well as from data regarding the birth of the first litter, these investigators concluded that the first estrus occurred on the average at 92.7 days after birth, ranging from 63 to 107 days. Vaginal introitus occurred on the average at 76.5 days after

¹ This investigation was supported by a grant from the Committee for Research in Problems of Sex, National Research Council.

² Present location, The Biological Laboratories, Harvard University, Cambridge, Massachusetts.

birth, ranging from 53 to 142 days. The authors point out that in the majority of the females the first ovulation was delayed approximately 5 days after vaginal introitus had occurred. They indicated further that in their strain of rats the first cycle was significantly longer than any of the succeeding ones.

From observations on the Wistar strain, King ('16) states that the first litters are dropped at about 3 months after birth, whereas gray Norway rats, living under natural conditions, do not begin to breed, as a rule, until they are at least 4 months of age (King, '39).

Freudenberger ('32), using vaginal introitus as an indicator for the onset of sexual maturity, reported that in the Wistar strain maturity occurred on the average at 46.9 days, ranging from 36 to 66 days after birth, whereas in the Long-Evans strain maturity was reached at 52.7 days, ranging from 39 to 101 days after birth.

Arai ('20), from histological studies of the ovaries of rats of the Wistar strain, came to the conclusion that ovulation occurred between 60 and 70 days after birth. Employing the same technique, Hargitt ('30) reported that albino rats became sexually mature approximately 40 to 45 days after birth.

Using the revolving cage method, in which the running activity of the animal was recorded, Slonaker ('24) reports that his animals became sexually mature at the average age of 80.6 days after birth, ranging from 63 to 109 days.

The work of Slonaker ('24), Freudenberger ('32), Blunn ('39), and others emphasizes that strain and dietary differences have an important bearing on the time of onset of puberty.

In the female rat direct observation of the copulatory response can be used as an accurate method to investigate the periodic changes in the estrous cycles of the non-pregnant and post-parturient animals without disturbing their cycles (Blandau, Boling and Young, '41; Blandau and Soderwall, '41). Observation of the copulatory behavior has distinct advantages in that (1) it permits one to ascertain the time of

the beginning and the end of heat, and (2) it provides a basis for determining the temporal relationship between heat and ovulation (Boling, Blandau, Soderwall and Young, '41).

None of the previous studies on pubertal female rats employed the copulatory response as a basis for ascertaining the time of sexual maturity. However, we have attempted by this method to determine more accurately: (1) the temporal relationship between vaginal introitus and the beginning of the first heat period; (2) the length of the first heat period in relation to that of succeeding periods; (3) the length of the first reproductive cycle and its comparison with subsequent cycles; (4) the time of the first ovulation and its relation to the beginning of the first heat, including the number of ova shed during the first heat as compared with the number shed in later periods; and (5) the number of females which became impregnated by either matings or artificial insemination during the first heat period.

MATERIALS AND METHODS

The data were obtained from 215 female rats all born within an interval of 4 days. The animals were members of a vigorous albino strain of Wistar extraction, which has been maintained at Brown University for a number of years. In order to standardize conditions as much as possible, all of the male young were discarded and each mother was allowed to suckle six female young. The females were weaned at 21 days of age and were then maintained on a ration of Rockland rat diet supplemented by a green vegetable fed twice each week. Water was provided *ad libitum*. Beginning at the time of weaning, the females were examined both morning and evening for vaginal introitus and for the copulatory response. When the first female showing signs of either vaginal canalization or manifestations of heat, a new routine of observations was substituted. This consisted of examination of the entire colony at hourly intervals, day and night, with respect to both vaginal introitus and the signs of heat. In order to reduce the influence of constant illumination on the cycle (Browman,

'37; Hemmingsen and Krarup, '37; Fiske, '41), the animals were kept in an ordinary room in which there was the normal alternation of daylight and darkness. The only light during the night observations and the taking of records was provided by a small shaded bulb attached to a movable crane which was so arranged that only one cage at a time was illuminated.

The onset of heat was ascertained by the female's response to manual manipulation as previously described by Blandau, Boling and Young ('41). That a female was about to come into heat could invariably be foretold by the marked tumescence and discoloration of the vaginal orifice. These changes in the vaginal orifice occur several hours before sexual receptivity becomes apparent.

Ninety-two females were used to determine the temporal relationship between the first heat and ovulation. Fifty-two of these, in which ovulation had been completed, served for ascertaining the number of ova shed during the first heat period. Forty-one females, selected at random, were examined for seven consecutive reproductive cycles. Thirty of these were sacrificed at the end of the eighth heat period in order to compare the number of ova shed in the first cycle with the number shed in the more mature animal. The ovaries of all of the experimental animals were removed and fixed in Bouin's fluid. They were then dehydrated in dioxan, embedded in paraffin, sectioned at 10μ and stained in Delafield's hematoxylin and eosin.

Fifteen females failed to give the copulatory response, even though the appearance of their vaginal orifices was that of an animal about to come into heat. The ovaries of these animals were subsequently removed and studied.

Thirty females were mated with mature males during the first heat period and ejaculations observed. To witness actual ejaculation is of importance because of the discrepancy in size between the mature male and the pubertal female—a discrepancy which makes failure of insemination possible.

Thirty-seven females were inseminated artificially during the first heat period according to the technique described by

Blandau and Jordan ('41). Both the mated and inseminated females were subsequently observed for pregnancy and birth of the young. At the termination of pregnancy, the animals were killed and the ovaries examined macroscopically to make certain that pregnancy was the result of the first ovulation.

The completion of the investigation would not have been possible without the generous assistance given by Dr. John R. Ring and Miss Catherine Fales in the continuous day and night observations of the animals.

OBSERVATIONS

The chronological relationship of vaginal opening to the first heat period in 184 females is illustrated in figure 1. It will be noted that, although there is a relatively close cor-

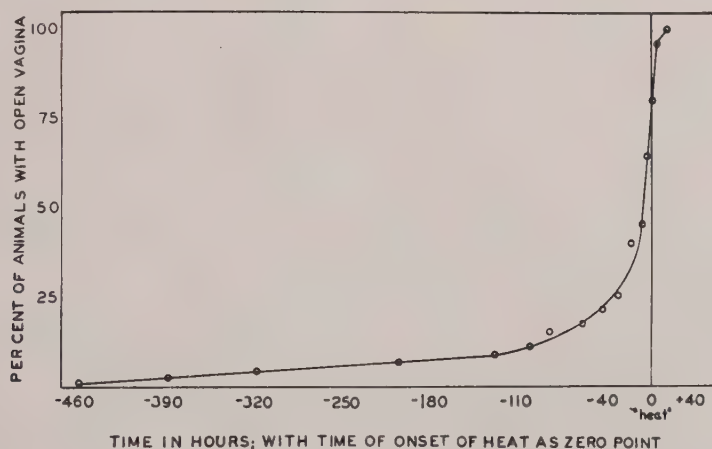


Fig. 1 Chronological relation of vaginal opening to first heat in 174 female rats.

relation between the onset of the first heat period and vaginal introitus in a large number of the females, there is, nevertheless, a relatively wide distribution in the time of vaginal opening in a significant number of individuals. Vaginal introitus and the onset of heat took place simultaneously in twenty-five animals. In twenty-six females, opening occurred from 1 to 12 hours after the onset of heat, and, in one animal, opening did not occur until 72 hours after the onset of heat.

Thus, a few females may be sexually receptive, yet their vaginal membranes may remain unruptured at least during the first hours of heat. In seventy-three females, opening occurred between 1 and 48 hours before the beginning of heat, and in the remaining females the time of opening varied from 48 to 459 hours before heat began.

Matters and MacKay ('34), in determining the time of sexual maturity in the black-hooded rat by histological examination of the ovaries at intervals after vaginal introitus, found that, though vaginal introitus may have occurred as early as 43 days of age, ovulation did not take place until approximately 70 days of age.

We emphasize that the beginning of heat, rather than vaginal introitus, is a truer index of the appearance of sexual maturity in the rat, because it is more closely associated with the time of ovulation and the ability of the animal to reproduce. Vaginal introitus alone has only relative significance in determining sexual maturity in this animal. Long and Evans ('22) have further emphasized this point by showing that in females in which the ovaries are removed at about the thirtieth day after birth the vaginal orifice is established at about the usual time.

The age of onset of the first heat period in 200 animals varied from 37 to 67 days, and showed a standard deviation of 5.46 days and an average of 49.4 days. No similar data, based upon the same method, are available in other strains for comparison. However, using the time of vaginal opening as a basis for determining the onset of sexual maturity, Freudemberger ('32) found that vaginal introitus occurred in the Wistar strain on the average of 46.9 days, ranging from 36 to 66 days after birth. Similar results were obtained by Blunn ('39). By using the same method as Freudemberger on the Long-Evans strain, he concluded that vaginal introitus took place, on the average, at 39 days, ranging from 34 to 45 days. Yet, as already emphasized, it must be remembered that vaginal opening is not necessarily concomitant with heat and ovulation.

The length of the first heat period in 138 females, which includes the first period in the forty-one females examined over consecutive cycles, averaged 9.07 hours, ranging from 1 to 20 hours, and showing a standard deviation of 4.08 hours. This average is approximately 5 hours shorter than the average length of heat (13.7 hours) previously reported for 609 heat periods observed in 114 mature females (Blandau, Boling and Young, '41). It will be shown later that with the onset of the second reproductive cycle the length of heat increases and becomes equal to the average length of heat in the mature female.

It was observed in a number of animals that the copulatory response of the first heat period does not begin as abruptly as it does in the succeeding heat periods. Also the intensity of the response is not as great in the pubertal female as in the mature animal. In nine animals "intermittent" estrous periods were observed during the first heat period, while none was observed in a group of forty-one animals examined during the first seven succeeding cycles. "Intermittent" estrus is not, however a unique characteristic of the first heat period, since it has been observed previously in a number of mature individuals (Blandau, Boling and Young, '41).

Observations were made on forty-one animals throughout the first seven consecutive estrous cycles in order to determine whether or not the lengths of the heat periods or the lengths of the successive reproductive cycles were altered as the cycles became established (tables 1 and 2). The length of the 328 heat periods averaged 13.4 hours, ranging from 3 to 24 hours. This compares favorably with the average figure of 13.7 hours obtained earlier on mature females of the same strain.

Upon analyzing the varying lengths of consecutive heat periods for each rat in order to test the hypothesis that there is a zero mean difference between these heat periods, it was found that the length of the first period shows a highly significant difference from the following seven heat periods. Beginning with the second period the mean length of heat remains

TABLE 1

The length of heat in hours in rats observed over the first eight heat periods.

ANIMAL	1st	2nd	3rd	4th	5th	6th	7th	8th	MEAN
5	8	12	11	7	11	9	12	11	10.1
12	14	16	8	16	18	17	13	15	14.6
13	7	15	13	12	13	11	8	11	11.2
14	13	10	12	14	10	9	12	14	11.7
15	11	18	16	18	13	13	16	17	15.2
18	8	14	11	13	16	12	14	12	12.5
20	6	13	10	14	12	11	9	9	10.5
25	7	16	8	11	13	14	10	11	11.2
28	7	15	11	16	10	13	18	13	12.8
30	8	15	13	15	24	6	8	14	12.8
34	5	15	13	15	14	15	17	15	13.6
37	11	13	18	19	16	17	15	14	15.3
41	6	10	12	15	15	16	16	12	12.7
43	11	11	17	12	14	16	11	13	13.1
44	7	13	15	10	15	15	13	12	12.5
55	12	14	17	18	16	11	13	12	14.1
57	10	16	13	16	17	12	7	17	13.5
59	17	17	16	17	11	13	14	16	15.1
69	6	14	10	9	8	9	11	14	10.1
75	6	21	16	20	15	18	17	18	15.3
76	11	13	10	10	15	13	11	11	11.7
89	8	16	12	12	16	16	17	18	14.3
90	10	12	20	17	20	17	15	16	15.8
106	16	3	8	18	18	16	18	19	14.5
141	8	10	17	11	17	16	17	10	13.2
147	11	13	11	12	6	7	10	8	9.7
148	6	18	18	15	14	11	13	17	14.0
154	8	10	14	13	14	14	14	14	12.6
170	11	10	11	11	14	16	13	14	12.5
178	15	16	16	14	19	16	14	15	15.6
192	7	14	9	11	7	10	11	13	10.2
193	15	15	15	22	15	15	14	13	15.5
200	4	15	15	16	18	10	9	7	11.7
201	11	16	15	18	14	11	15	14	14.2
205	12	9	16	16	13	13	14	15	13.5
206	9	20	13	14	16	14	12	13	13.8
238	9	13	17	16	15	17	14	18	14.8
243	10	14	13	15	13	17	15	14	13.8
253	13	7	18	20	21	17	19	20	15.8
257	13	17	14	21	14	15	12	11	14.6
261	14	18	17	21	12	14	17	18	16.3
Mean	9.78	13.8	13.6	14.9	14.4	13.5	13.4	13.9	13.4
σ	3.10	3.45	3.89	3.52	3.55	3.05	2.97	3.00	

relatively constant throughout the consecutive reproductive cycles (table 1). The mean length of heat in cycles 2 to 8 compares favorably with that previously reported (13.7 hours) for mature animals (Blandau, Boling and Young, '41).

When the mean lengths of the first seven consecutive reproductive cycles are examined (table 2), it is noted that the mean length of the first cycle is at least twice that of any of the succeeding six cycles. The second cycle also shows a significant difference from the following five.

TABLE 2
The mean length of consecutive reproductive cycles.

CYCLE	NUMBER OF ANIMALS	MEAN LENGTH IN HOURS	STANDARD DEVIATION	RANGE
1st	41	221.0	67.3	115 to 306
2nd	41	123.5	28.2	71 to 191
3rd	41	113.5	18.7	69 to 170
4th	41	112.3	24.8	83 to 194
5th	41	108.6	19.7	72 to 167
6th	41	111.3	20.2	69 to 169
7th	41	104.1	19.5	65 to 167

The data in table 2 (from cycle 2 through 7) compare favorably with those previously reported for 567 cycles in mature females of the same strain, in which the mean lengths of consecutive reproductive cycles varied from 99.2 to 105.2 hours (Blandau, Boling and Young, '41). The data are also in agreement with the findings of Long and Evans ('22) in which they observed that the first cycle, as determined by the smear, was distinctly longer than the succeeding cycles.

The general picture of the length of the first heat period and cycle, compared to succeeding heat periods and cycles, indicates that the attainment of normal reproductive rhythmicity is a gradual process, but that once the hormonal mechanism is more thoroughly established, other factors being equal, the animal will display a considerable consistency of behavior.

An hourly record of the time of day at which the first heat began in 200 females is shown in table 3. A comparison of these

data with those previously reported by Blandau, Boling and Young ('41) indicates that the majority of their mature females came into heat between 4 P.M. and 1 A.M., while the majority of our pubertal animals (165) came into their first heat between 7 P.M. and 5 A.M. The data on the time of onset of heat in cycles 2 through 7, in the forty-one females examined in consecutive cycles, correspond more nearly to the time of onset of heat in mature females in that the majority of these animals also came into heat between 4 P.M. and 1 A.M.

Since it is generally assumed that ovulation does not take place before the establishment of the vaginal orifice, and because there are no data in the rat on the temporal relationships between vaginal introitus, the first heat, and ovulation, an investigation of these relationships seemed desirable.

TABLE 3
Hourly record of the time of day at which heat was detected.

TIME OF DAY	NUMBER OF CASES	TIME OF DAY	NUMBER OF CASES	TIME OF DAY	NUMBER OF CASES
12 M.	1	8 P.M.	9	4 A.M.	11
1 P.M.	0	9 P.M.	18	5 A.M.	13
2 P.M.	0	10 P.M.	11	6 A.M.	7
3 P.M.	0	11 P.M.	19	7 A.M.	5
4 P.M.	1	12 P.M.	15	8 A.M.	0
5 P.M.	6	1 A.M.	13	9 A.M.	1
6 P.M.	2	2 A.M.	33	10 A.M.	2
7 P.M.	8	3 A.M.	23	11 A.M.	2

Table 4 summarizes the temporal relationship between the onset of the first heat and ovulation for ninety-two animals. In only one of eight animals examined between 3 and 4 hours after the beginning of heat had ovulation occurred. By the tenth hour after the beginning of heat, ovulation was in progress or had been completed in 100% of the animals. When these data are compared with similar data for mature females (Boling, Blandau, Soderwall and Young, '41), it is apparent that ovulation is initiated somewhat earlier during the first heat period. The suggestion is offered that the shorter ovulation time is directly related to the shortened total length of the first heat period.

If ovulation can be said to occur sometime between 5 and 10 hours after the beginning of heat, it is apparent that a relatively small number of females ovulate before vaginal introitus occurs (fig. 1).

The ovaries of all of the animals used in determining the time of ovulation were examined microscopically in order to make certain that only one group of eggs had ovulated.

Figure 2 is a typical example of the appearance of an ovary removed 5 hours after the beginning of the first heat period. Since there are no corpora lutea present from previous ovulations, the ovary as a whole appears to be relatively small. The center of the ovary is filled with a large amount of loose

TABLE 4
Temporal relationship of heat and ovulation.

	HOURS AFTER THE BEGINNING OF HEAT						
	3-4	4-5	5-6	6-7	7-8	8-9	9-12
Number of animals	8	14	16	8	12	17	17
Number in which ovulation had begun or was completed	1	7	9	6	10	14	17

connective tissue, and the maturing follicles bulge around the periphery. Other sections show a number of follicles, both large and small, undergoing atresia. The preovulatory follicular growth is progressing rapidly. The cells of the cumuli are undergoing separation and in several follicles the ova, surrounded by their cumuli, are floating free in the antra. The secondary follicular fluid is making its appearance and there is evidence of the infolding of the granulosa with subsequent "pocketing." In all the ova the first polar bodies had formed and the second maturation spindles were being organized.

Figure 3 is a fortunate section through one of eight follicles in an animal killed 7 hours after the beginning of the first heat. The rupture area is just breaking loose and the ovum with the surrounding cumulus and follicular fluid can be seen

streaming toward the rupture point. The first polar body is formed and the second maturation spindle is completed.

Figures 4, 6 and 7 are sections through freshly ruptured follicles from ovaries removed 10 hours after the beginning of heat. In figures 4 and 6 the ova surrounded by their cumuli are adjacent to the point of rupture. The tension lines in the follicular fluid at the time of rupture are clearly visible.

The observations of Minot (1891) on the guinea pig, and Hammond ('14) on pigs and rabbits, showed that fecundity is low during the early life of the female. The fewer offspring born to young animals is due, according to Hammond, to the smaller number of ova shed.

On the other hand, in swine, McKenzie ('28) found that early breeding increased the litter size throughout the reproductive life of the animal.

Since it is possible to determine with considerable accuracy the time of the first ovulation, we were interested in comparing the number of ova shed during the first ovulation with the number ovulated after at least six cycles had elapsed. In addition, data have been obtained on matings and artificial inseminations made during the first heat period.

In fifty-two females killed at the end of the first heat period the average number of ova recovered from the periovarial sacs was 7.8 ova per animal, ranging from 5 to 12. Thirty females were killed at the end of heat after at least six reproductive cycles had elapsed. The average number of ova recovered from these sacs was 9.2 per animal, ranging from 6 to 13.

Of the thirty females in which observed matings were made, twenty-one or 70% became impregnated. The average litter size was 6.0, ranging from 3 to 10. Thirty or 81% of the thirty-seven females artificially inseminated during the first heat period were impregnated. The average litter size in this group was 6.5, ranging from 4 to 11. The average number of corpora lutea observed macroscopically on the surface of the ovaries of both mated and inseminated females was 8.5. In the majority of cases parturition was observed. The only

respect in which parturition in young animals differs from that of the mature animal is the greater tendency of the young mother to kill and eat the young during and immediately following parturition.

Fifteen females, in which the vaginal membranes ruptured, failed to come into heat. In all of these animals vaginal tumescence was obvious at varying intervals during the period of observation. The ovaries were subsequently removed and examined. Figure 5 is a section of an ovary removed from one of these females 15 hours after the first vaginal tumescence appeared. Five fresh corpora lutea are seen in the section and five fresh ova were recovered from the periovarial sac. Ovulation was definitely of recent occurrence. The ovaries of eight of the fifteen animals revealed at least two sets of corpora lutea, indicating that several ovulations had taken place without the females coming into active heat. In six females the sections revealed only one set of corpora lutea and follicles of varying sizes.

A number of females, in which vaginal tumescence occurred without heat, were placed with vigorous males. Numerous mating attempts, without intromission, were observed.

DISCUSSION

The time of attainment of sexual maturity in the female rat may be defined as the earliest period at which the animal is capable of being impregnated. Maturity involves the development of the genital tract to a point at which vaginal introitus, sexual receptivity and ovulation occur in the proper sequence so that fertilization and implantation are possible.

From the data which have been presented it appears that in the majority of females the temporal relationship between the onset of vaginal introitus, heat and ovulation is correlated in such a way that normal reproduction is not delayed. However, we are in agreement with the conclusion of Long and Evans ('22) that it would be a mistake to regard any single phenomenon of puberty as a wholly reliable criterion of normal reproductive ability. These authors were of the opinion that

in the majority of instances where vaginal introitus and the first heat occur simultaneously, the vaginal membrane does not rupture until estrus proper has ensued. In only a very few animals, in the present investigation, was vaginal introitus delayed until near the end of sexual receptivity or longer.

The possibility exists that in some females the shortened length of heat may not provide a sufficient opportunity for mating, especially if sexual receptivity is of low intensity. In the present series low intensity of sexual receptivity was observed only during the first heat period. In the subsequent heat periods the intensity of elicitable copulatory responses was in every respect comparable to that of mature animals.

It is commonly acknowledged that pregnancy may not occur in some females of an unselected group which is confined continuously with vigorous males. In guinea pigs, observed by Young, Dempsey, Myers and Hagquist ('38), the occasional failure of mating was attributed either to poor follicular development which resulted in failure of ovulation, or, in females in which ovulation occurred normally, to a high threshold to the conditioning action of estrogen.

In a more comprehensive study on the failure of mating in the rat (Boling, Blandau, Rundlett and Young, '41), it was revealed that in some females the ovaries appeared normal, although mating was not successful. In other animals the histological appearance of the ovaries indicated a deficiency in hypophyseal stimulation. When these abnormal females were tested for their sensitivity to estrogens, the conclusion was reached that the basic factor in the failure of mating was due to the decreased sensitivity to estrogens. That age is an important factor in the degree of response to estrogenic treatment is shown by the work of Hemmingsen ('33) and Wilson and Young ('41). According to Wilson and Young the female rat attains a high sensitivity to estrogen about the thirtieth day after birth, irrespective of the presence or absence of the ovaries and uterus since birth.

In females which do not come into heat the present experiments show that vaginal tumescence and ovulation nevertheless occur. One might conclude that a higher estrogen level is necessary to condition the animal for sexual receptivity than to call forth the remaining reproductive phenomena. Further emphasis is given this speculation by the observation that all of the ovaries removed during the first heat period showed normal follicular development, and ovulation was either in progress or had been completed. However, in the observations of Boling, Blandau, Rundlett and Young ('41), female rats were described in which follicular development was poor or abnormal but matings nevertheless occurred. Many similar observations on other species have led to the general conclusion that in the majority of animals heat and ovulation occur concomitantly. In certain individuals, however, heat is not dependent upon ovulation, nor ovulation upon the manifestation of sexual receptivity.

The comparative data on the number of ova shed during the first and sixth heat periods and on the matings and artificial inseminations made during the first heat period are to a certain extent in accord with those of Hammond ('14) for the sow and the rabbit, as well as those of Asdell, Bogart and Sperling ('41) for the rat. It is true that the number of ova shed during the first heat period is significantly lower than the number liberated in more mature females. However, the average litter size of rats mated or inseminated artificially during the first heat period does not vary greatly from those obtained by normal matings or artificial inseminations of mature females (King, '39; Blandau and Jordan, '41). In order to elucidate this point completely, it would be necessary to compare the number of corpora lutea found in the ovaries with the actual number of young born after first matings in a large number of females.

SUMMARY

Two hundred and fifteen albino female rats were observed from the time of weaning to sexual maturity. The onset of

sexual receptivity was used as a basis for the determination of the temporal relationships between vaginal introitus, heat and ovulation. In addition, data were obtained on (1) the length of the first period of heat and the first reproductive cycle compared with succeeding heat periods and cycles; (2) the time of day that the first period of heat began; (3) the number of ova shed during the first and sixth cycles; and (4) the effects of matings and artificial inseminations during the first period of heat.

1. The temporal relationship between vaginal introitus and the onset of the first heat in 174 females varied from 459 hours before heat began to 72 hours after the onset of heat. In the majority of animals vaginal introitus occurred from 48 hours before the onset of heat to 12 hours after heat began.

2. The average age for the onset of heat in 200 females was 49.4 days, ranging from 37 to 67 days, with a standard deviation of 5.46 days.

3. The length of the first heat period as observed in 138 females averaged 9.07 hours, ranging from 1 to 20 hours, with a standard deviation of 4.08 hours. Nine cases of "intermittant" estrus were encountered.

4. Forty-one females were observed throughout the first seven consecutive estrous cycles. The first heat period was significantly shorter than the succeeding periods, and the first and second reproductive cycles were significantly longer than the remaining cycles.

5. The first period of heat began in the majority of females between 7 P.M. and 5 A.M.

6. The first ovulation occurred between 5 and 10 hours after the onset of the first period of heat.

7. The average number of ova shed during the first heat period was 7.8, ranging from 5 to 12. The average number of ova recovered after at least six cycles had elapsed was 9.2, ranging from 6 to 18.

8. Seventy per cent of the thirty females mating during the first heat period were successfully impregnated. The average size of the litters was 6, ranging from 3 to 10. Eighty-one

per cent of thirty-seven females inseminated artificially bore litters which averaged 6.5, ranging from 4 to 11. The average number of corpora lutea for both groups was 8.5.

9. Fifteen females failed to become sexually receptive even though vaginal introitus and ovulation occurred.

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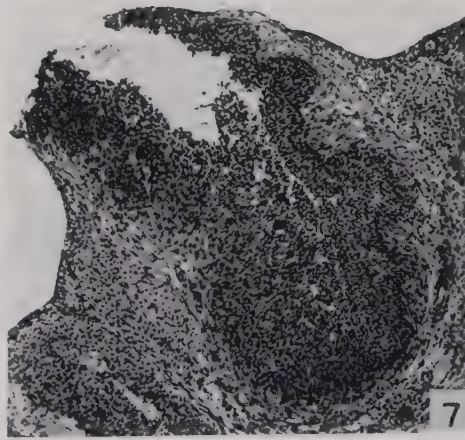
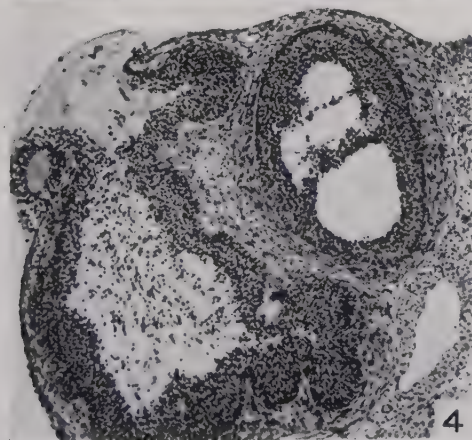
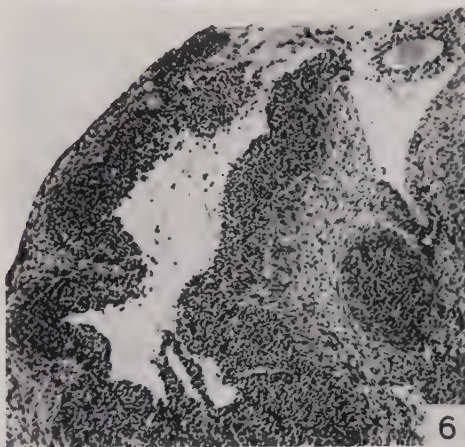
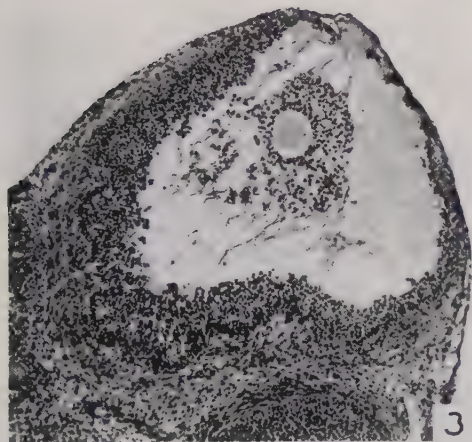
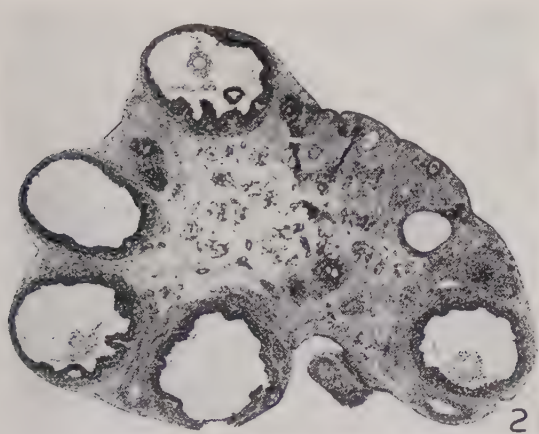
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PLATE 1

EXPLANATION OF FIGURES

- 2 Section through ovary removed 5 hours after the beginning of the first heat. $\times 18$.
- 3 Follicle in which ovulation is just beginning, removed 7 hours after the onset of the first heat. $\times 75$.
- 4, 6 and 7 Recently ruptured follicles removed 10 hours after the onset of the first heat. The extruded ova are still near the point of rupture in figures 4 and 6. $\times 75$.
- 5 Section through ovary removed 15 hours after the appearance of vaginal tumescence without subsequent heat. The condition of the five corpora lutea indicates an ovulation of recent occurrence. $\times 18$.



BOOK REVIEW

MANUALS OF EXPERIMENTAL EMBRYOLOGY

EXPERIMENTAL EMBRYOLOGY: A MANUAL OF TECHNIQUES AND PROCEDURES. Roberts Rugh; N. Y. University Bookstore, 18 Washington Place, N. Y. City, 1941; 303 pp. Wire-O-Binding.

A MANUAL OF EXPERIMENTAL EMBRYOLOGY. Viktor Hamburger; University of Chicago Press, 1942; 213 pp. Clothbound.

These two recently published manuals are welcome and useful contributions to the teaching of embryology in a phase in which all too few such aids are available. Research in experimental embryology has elucidated so much of fundamental significance to an understanding of the dynamic forces of development and the interaction of the factors of differentiation that this field cannot be overlooked by the modern student of embryology. Until the publication of these two manuals, a person desiring a working knowledge of the techniques of experimental embryology has been forced to glean such techniques in a fragmentary way from a widely-scattered literature. Now in these two volumes is brought together a wide array of significant and often by now classical experiments in this field. In addition the authors have made many helpful suggestions that often mean the difference between success and failure of students in their prosecution of these experiments.

Both authors have succeeded admirably in designing experiments that are at once critical in the analysis of embryological processes and yet are sufficiently simple in terms of materials and skills required that students may follow them. Each manual has the virtue of being based on the author's teaching experience and each represents essentially the material of courses taught by them in their respective institutions. Experiments outlined make use of living material, obtainable either locally or easily secured through purchase from supply houses. Most instruments used can be made by the students following directions and illustrations given. Experiments are arranged in

natural groupings, but in each case, they are treated as units, such that considerable latitude is permitted in the sequence in which they are carried out. Laboratory directions are explicit, supplemented in Hamburger's manual by drawings, in Rugh's manual by drawings and numerous excellent photographs. Particularly valuable are provisions for adequate controls and records, and precautions to the student against pitfalls to avoid, based on the author's experience. In both manuals, each experiment or group of related experiments is accompanied by a brief discussion of the literature and the theoretical implications of the experiment, followed by a bibliography.

After an introduction and a section on equipment, supplies and the making of special instruments, both manuals present an extensive list of experiments dealing with Amphibian and chick embryos, regeneration in *Planaria*, and axial gradient phenomena. Many of the same experiments are to be found in both volumes. For example, in both are to be found experiments dealing with ovulation and fertilization, parthenogenesis, effect of environmental factors, vital staining of presumptive areas, analysis of pregastrulation stages, transplantation in relation to self-differentiation and induction, parabiosis and similar aspects of Amphibian development. Both manuals present experiments involving chorio-allantoic grafts and transplantation in chick embryos. It should be emphasized, however, that the apparent similarity above ends with the subject matter and the end result sought, and that each manual's presentation reflects the particular approach and viewpoint of its author. Doctor Hamburger has more deliberately sought to arrange the experiments in topical sequence following the ontogeny of the organism. Also, in accordance with his own research interests, he has included considerably more material on chick experimentation than has Doctor Rugh. The latter, pursuant to his research interests in the same way, has included a greater number of experiments and with more detailed directions on Amphibia. He has added also a section on the laboratory rearing of the eggs of *Artemia*, *Daphnia*, *Tubifex* and *Enchytraea*, together with a section dealing with a variety of aquarium fishes, their care, breeding habits and embryonic development.

Both manuals carry complete drawings of the Shumway series of embryonic stages of *Rana pipiens*, the Pollister and Moore series of *Rana sylvatica* stages, and the R. G. Harrison series of *Ambystoma maculatum* Shaw (*A. punctatum* L.) stages. In addition, Rugh's manual carries a series of 207 photographs of normal *R. pipiens* embryos and ninety-five photographs of abnormal embryos of the same species, such as may be encountered in the course of experimentation.

Both manuals are excellent contributions of skilled investigators and teachers in this rapidly developing field of experimental embryology. Each has much to recommend it.

Many noteworthy items must remain unmentioned in this review for lack of space, but which the interested reader may observe for himself. In the writer's opinion, particularly helpful features of Hamburger's manual are the logical arrangement of experiments in relation to the phases of embryology involved, the general format of the book, and the index. In the same way, Rugh's manual is particularly outstanding in its more elaborate detail of procedures, methods, etc., the profusion of its excellent photographic illustrations throughout, and its twenty-five page glossary of terms.

With either or both of these manuals in hand, the enquiring student will find an abundance of challenging subject matter, and many avenues by which to approach the hinterlands of the unknown in experimental embryology.

JOHN W. PRICE
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BOOK REVIEW

THE GENETICS OF THE MOUSE. By HANS GRÜNEBERG. Published by Cambridge University Press, London, New York, Toronto, Bombay, Calcutta, Madras, 1943. 397 pages, 43 illustrations. Price \$7.00.

This is a scholarly, comprehensive treatise in which the author has skillfully organized with their genetic analyses the results of research on the mouse. The author demonstrates that work with genetically controlled groups of mice is a method of research. Geneticists have made available for experimental investigation, populations possessing pathological entities similar to those found in the human being. The large group of genetic abnormalities and variations surveyed indicates that the mouse is indeed a choice animal for medico-biologic research. This book contains information that should be available to all investigators using the mouse as an experimental animal. It is surprising that such a large amount of material drawn from such widely divergent sources could be so effectively concentrated in a small volume.

After a brief discussion of the subjects of taxonomy, reproduction and general growth a review of the literature on "The Inherited Differences" is given. This section takes up 258 of the 397 pages. All portions of this review are interestingly written in a crisp and clear style with extensive but not burdensome reference to the original papers. The text is not a mere recitation of the findings of various investigators. Clear definition of problem and purpose is given. Subjects covered include genetic factors controlling coat color, endocrine studies of a genetic character (pituitary dwarfism, adrenal and genital aberrations), abnormalities of the brain and sense organs with a good discussion of the papers reporting anomalous embryonic development, genetically controlled anemias of the mouse in a portion on the blood and blood-forming organs, skeletal and dental pathology associated with the gray lethal factor and other anomalies such as posterior duplication and congenital absence of the tibia in those pages devoted to the skeleton, cleft palate in a discussion of the alimentary tract. A few pertinent suggestions for the course of future research on the mouse are given in connection with discussion of the urogenital system. It is pointed out that in order to analyze genetically diseases of the kidney and other organs, mice must be observed for their entire life span since so many mouse diseases appear in middle and late life.

After presentation of this review of research the findings are classified. The author feels that as a result of gene action normal development can be suppressed (or remain incomplete), be exaggerated, or deflected in the wrong direction, or there may be degeneration. Suggestions for obtaining genetic variations for future investigations are given. Under the title of "Quantitative Differences" genetic and non-genetic factors affecting litter-size, body weight and size are presented. Genetics of blood serology and physiological strain differences such as behavior, water requirements, hypo and hyperglycemia and resistance to infectious diseases are presented next. Chromosomal inheritance, induced genetic changes and the genetics of cancer are discussed in separate sections. The section on cancer was written by C. C. Little and P. A. Gorer and is a concise summary of the findings to date in experimental cancer as it concerns the mouse. With the great extension of investigations on mouse cancer this chapter is an appropriate one. It achieves the same degree of excellence as the remainder of the book. There is a short section on the keeping and breeding of mice. A bibliography of 1141 references classified according to subject is appended.

The book should especially interest those who combine the medical and biological points of view in research on the mouse. Doctor Grüneberg has taken the attitude that the whole body of medical knowledge can be concentrated on a study of clinical conditions in the mouse. In this way the field of developmental genetics should advance tremendously. As is well indicated in the book, one of the main contributions of the study of the mouse should be to indicate the genetic influence not only upon the anatomy but upon embryology, physiology and pathology of the mouse, especially in those instances where the resemblance to human entities is so striking.

ARTHUR KIRSCHBAUM,
University of Minnesota

APPROVED

MOTION PICTURE FILMS

The following films have been approved by one or more members of the Review Committees of the American Association of Anatomists or the American Society of Zoologists, and The Wistar Institute:

SUBJECT AND TITLE	LENGTH IN REELS (1-16 MM. REEL = 400 FT. RUNNING TIME ABOUT 15 MIN.)		PRODUCER	CONDITIONS FOR SECURING FILMS	DISTRIBUTOR
ANATOMY					
Congenital anomalies (monochrome, silent) (kodachrome, silent)	1	J. Sarnoff	Sale: Monochrome, \$25.00 Kodachrome, \$75.00	Sarnoff Motion Picture Film Library 1406 Albemarle Road Brooklyn, N. Y.	
* The management of cica- tricial strictures of the esophagus. Treatment of cancer of the esopha- gus. Bronchoscopy with insufflation of sulfanil- amide in chronic bronchiectasis (kodachrome, silent)	1	E. H. Ingersoll	Rental, \$8.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.	
* Surgical anatomy (kodachrome, silent)	4	C. J. Baumgartner	Sale Kodachrome	Billy Burke Productions 7416 Beverly Blvd. Hollywood, Calif.	
BLOOD CELLS					
* 10. Normal and abnormal white blood cells in tissue cultures (monochrome, silent)	1	W. H. Lewis M. R. Lewis A. R. Rich M. M. Wintrobe	Rental, \$4.00 per day Sale, \$25.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.	
CANCER CELLS					
* 6. Dividing cancer cells in vitro (monochrome, silent)	160 ft.	W. H. Lewis M. R. Lewis	Rental, \$2.00 per day Sale, \$10.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.	
* 3. Tumor cells and macro- phages in tissue cul- tures. Rat sarcomas and carcinomas (monochrome, silent)	360 ft.	W. H. Lewis	Rental, \$3.50 per day Sale, \$20.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.	
CIRCULATION					
* The control of small blood vessels (mono- chrome, silent)	1	G. P. Fulton B. R. Lutz	Rental or sale	Dr. G. P. Fulton College of Liberal Arts Boston University Boston, Mass.	

SUBJECT AND TITLE	LENGTH IN REELS (1-16 MM. REEL = 400 FT. RUNNING TIME ABOUT 15 MIN.)	PRODUCER	CONDITIONS FOR SECURING FILMS	DISTRIBUTOR
<i>Circulation (Cont.)</i>				
* Effect of various drugs on contractions of mesenteric lymphatics in the rat (monochrome, silent)	300 ft.	R. L. Webb	Rental, \$3.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* The heart and circulation (monochrome, silent)	1	A. J. Carlson	Sale	Erpi Classroom Films, Inc. 35-11 35th Ave. Long Island City N. Y.
* Mesenteric lymphatics, their conduct and the behavior of their valves in the living rat (monochrome, silent)	1	R. L. Webb	Rental, \$4.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* Micromanipulative studies of blood capillaries (monochrome, silent)	1	B. W. Zweifach	Rental, \$4.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* 7. Growth of capillaries and endothelium from the sub-cutaneous tissue of 7-day chick embryos in 2-day cultures in Locke-bouillon-dextrose medium (monochrome, silent)	140 ft.	W. H. Lewis	Rental, \$2.00 per day Sale, \$9.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
CYTOLOGY				
* 8. Pinocytosis. Drinking by cells (monochrome, silent)	264 ft.	W. H. Lewis	Rental, \$3.00 per day Sale, \$16.50	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* 9. Dividing normal adult rat fibroblasts in vitro (monochrome, silent)	160 ft.	W. H. Lewis	Rental, \$2.00 per day Sale, \$10.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
EMBRYOLOGY				
* Activity of heart muscle, from rat embryos, grown in tissue culture (monochrome, silent)	1	C. M. Goss	Rental, \$4.00 Sale, \$25.00	Dr. C. M. Goss University of Alabama University, Ala.
Development of a salamander (monochrome, silent)	1	L. S. Stone and T. C. Kramer	Rental Sale	Films, Inc. 330 W. 42nd St. New York, N. Y.
* 1. The early development of the rabbit egg in vitro (monochrome, silent)	1	W. H. Lewis and P. W. Gregory	Rental, \$4.00 per day Sale, \$25.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* 4. The early development of the rabbit egg (short film — selected scenes from the long film) (monochrome, silent)	80 ft.	W. H. Lewis and P. W. Gregory	Rental, \$1.50 per day Sale, \$5.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.

SUBJECT AND TITLE	LENGTH IN REELS (1-16 MM. REEL = 400 FT. RUNNING TIME ABOUT 15 MIN.)	PRODUCER	CONDITIONS FOR SECURING FILMS	DISTRIBUTOR
<i>Embryology (Cont.)</i>				
* 5. Early divisions, 2-8 cells, of living monkey egg in vitro (monochrome, silent)	80 ft.	W. H. Lewis and C. G. Hartman	Rental, \$1.50 per day Sale, \$5.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
Forming an egg (kodachrome, sound)	1	D. C. Warren and H. M. Scott	Rental, \$3.00 per day	Poultry Department Kansas State College Manhattan, Kans.
* Where chick life begins (kodachrome, silent)	1	A. L. Romanoff	Rental Sale	Ralston Purina Co. St. Louis, Mo.
* 11. Development of Zebra fish egg (monochrome silent)	1	W. H. Lewis and E. C. Roosen-Runge	Rental, \$4.00 per day Sale, \$25.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* Development of the opossum (kodachrome silent)	150 ft.	E. J. Farris	Rental, \$3.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
GENERAL				
Practical preventive orthodontics (kodachrome silent)	2	M. J. Futterman	Rental	Dr. M. J. Futterman 1749 Grand Concourse Bronx, N. Y.
* High speed motion pictures taken with stroboscopic light (monochrome, silent)	1	H. E. Edgerton K. J. Germeshausen H. E. Grier	Rental free Sale, \$25.00	Prof. Charles E. Locke Massachusetts Institute of Technology Cambridge, Mass.
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* Unseen worlds (monochrome, sound)	1	RCA Victor Camden, N. J.	Transportation charges only	Wm. J. Ganz Co. 19 E. 47 St. New York, N. Y.
NERVOUS SYSTEM				
* Effect of electrical stimulation of the right ansiform lobe of the cerebellum of the cat (<i>Felis domestica</i>) (monochrome, silent)	100 ft.	S. L. Clark	Rental	Dr. Sam L. Clark Department of Anatomy Vanderbilt University Nashville, Tenn.
* The eyes of science (monochrome, silent)	3	Bausch & Lomb Optical Co.	Rental free	Bausch & Lomb Optical Co. Rochester, N. Y.
* The nervous system (monochrome, silent)	14	I. V. Pavlov and others	Rental or sale	Brandon Films, Inc. 1600 Broadway New York, N. Y.

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REPRODUCTION				
* The biology of reproduction in rats (kodachrome, silent)	1	E. J. Farris	Rental, \$8.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* Maternal behavior in the rat (monochrome, silent)	350 ft.	F. A. Beach	Rental, \$8.00 per day Sale, \$30.00	American Museum of Natural History New York, N. Y.
Mating behavior in the leopard frog, <i>Rana</i> <i>pipiens</i> (monochrome, silent)	250 ft.	F. A. Beach	Rental, \$8.00 per day Sale, \$20.00	American Museum of Natural History New York, N. Y.
Ovulation in the frog, <i>Rana pipiens</i> (mono- chrome, silent)	512 ft.	R. Rugh	Rental, \$5.00 per day Sale, \$25.00	Dr. Roberts Rugh N. Y. University Washington Square College New York, N. Y.
* Proestrous and oestrous behavior in the guinea pig (monochrome, silent)	150 ft.	W. C. Young E. W. Dempsey R. Hertz and H. L. Myers	Rental, \$1.50 per day Sale	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
A rapid pregnancy test (kodachrome, silent)	250 ft.	U. J. Salmon and others	Rental free	Dr. U. J. Salmon 875 Fifth Avenue New York, N. Y.
* Studies in human fertility	1 (2000 ft.)	Audio Produc- tions, Inc.	Rental free	Ortho Products, Inc. Linden, N. J.
RESPIRATION				
Mechanisms of breathing (monochrome, sound)	1	V. Johnson	Sale	Erpi Classroom Films, Inc. 35-11 35th Ave. Long Island City N. Y.

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H. E. Jordan	Chauncey Juday
C. E. McClung	H. W. Norris
George Lefevre	C. E. McClung
T. G. Lee	H. F. Nachtrieb

AMERICAN SOCIETY OF ZOOLOGISTS (AMALGAMATED)

	<i>President</i>	<i>Vice-President</i>	<i>Executive Committeemen</i>
1914.	C. E. McClung	M. F. Guyer	H. E. Jordan—1 year
1915.	W. A. Loey	W. E. Ritter	H. F. Nachtrieb—2 years
1916.	D. H. Tennent	Charles Zeleny	H. V. Wilson—3 years
1917.	M. M. Metcalf	Charles Zeleny	George Lefevre—4 years
1918.	George Lefevre	L. L. Woodruff	A. F. Shull—5 years
1919.	C. M. Child	H. H. Wilder	D. H. Tennent—5 years
1920.	Gilman A. Drew	Caswell Grave	R. P. Bigelow—5 years
1921.	Charles A. Kofoid	Aaron T. Treadwell	L. J. Cole—4 years
1922.	H. H. Wilder	Bennet M. Allen	H. V. Wilson—5 years
1923.	M. F. Guyer	Robert A. Budington	M. M. Metcalf—5 years
1924.	R. G. Harrison	R. K. Nabours	George Lefevre—5 years
1925.	C. R. Stockard	C. R. Moore	C. M. Child—5 years
1926.	S. O. Mast	W. C. Allee	G. A. Drew—5 years
1927.	S. J. Holmes	Robert Chambers, Jr.	C. A. Kofoid—5 years
			H. H. Wilder—5 years
			J. H. Gerould—1 year
1928.	Caswell Grave	M. H. Jacobs	M. F. Guyer—5 years
1929.	C. B. Davenport	Fernandus Payne	R. G. Harrison—5 years
1930.	H. V. Neal	E. E. Just	C. R. Stockard—5 years
1931.	Fernandus Payne	D. E. Minnich	S. O. Mast—5 years
1932.	W. C. Curtis	L. V. Heilbrunn	S. J. Holmes—5 years
1933.	Charles Zeleny	J. H. Bodine	Caswell Grave—5 years
1934.	A. H. Sturtevant	H. W. Rand	C. B. Davenport—4 years
1935.	R. W. Hegner	Sewall Wright	H. V. Neal—5 years
1936.	W. C. Allee	C. W. Metz	Fernandus Payne—5 years
1937.	F. L. Hisaw	Helen D. King	W. C. Curtis—5 years
1938.	M. H. Jacobs	T. S. Painter	Charles Zeleny—5 years
1939.	J. T. Patterson	H. B. Goodrich	A. H. Sturtevant—5 years
1940.	W. R. Coe	D. H. Wenrich	Robert W. Hegner—5 years
1941.	R. E. Coker	J. P. Visscher	W. C. Allee—5 years
1942.	L. L. Woodruff	C. G. Hartman	F. L. Hisaw—5 years

Secretary-Treasurer

1914–1918.	Caswell Grave	1918–1921.	W. C. Allee
	<i>Secretary</i>		<i>Treasurer</i>
1921–1924.	W. C. Allee	1922.	D. H. Tennent
1925–1928.	D. E. Minnich	1923–1924.	R. H. Bowen
1928–1929.	L. B. Arey	1925–1930.	L. B. Arey
1929–1930.	D. E. Minnich	1930–1933.	A. B. Dawson
1930–1933.	W. H. Cole	1933–1935.	B. H. Willier
1933–1936.	H. B. Goodrich	1935–1938.	W. N. Hess
1936–1939.	E. G. Butler	1938–1941.	T. C. Nelson

*Representatives in the Division of Biology and Agriculture,
National Research Council*

F. R. Lillie 1919-1923	E. G. Conklin 1923-1926	H. S. Jennings 1929-1931
G. H. Parker 1919-1922	J. T. Patterson 1924-1927	L. C. Dunn 1931-1932
M. F. Guyer 1919-1921	L. L. Woodruff 1925-1928	J. H. Bodine 1932-1935
William Patten 1921-1924	E. G. Conklin 1926-1929	L. V. Heilbrunn 1935-1938
H. S. Jennings 1922-1925	G. H. Parker 1926-1929	Laurence Irving 1938-1941

Associate Editors of the Journal of Morphology

1921.	Gary N. Calkins	J. S. Kingsley	William Patten
1921-1922.	E. G. Conklin	M. F. Guyer	W. M. Wheeler
1921-1923.	C. A. Kofoid	F. R. Lillie	J. T. Patterson
1922-1924.	G. A. Drew	H. V. Neal	L. L. Woodruff
1923-1925.	Caswell Grave	D. H. Tennent	H. V. Wilson
1924-1926.	Helen Dean King	S. O. Mast	A. A. Schaeffer
1925-1927.	R. W. Hegner	Fernandus Payne	H. B. Ward
1926-1928.	L. J. Cole	J. H. Gerould	M. H. Jacobs
1927-1929.	W. C. Curtis	J. Percy Moore	A. F. Shull
1928-1930.	Edgar Allen	G. A. Baitzell	R. E. Coker
1929-1931.	S. R. Detwiler	R. S. Lillie	H. J. Muller
1930-1932.	W. R. Coe	E. N. Harvey	H. E. Jordan
1931-1933.	L. V. Heilbrunn	S. O. Mast	G. K. Noble
1932-1934.	D. E. Minnich	B. H. Willier	L. L. Woodruff
1933-1935.	L. R. Cleveland	F. L. Hisaw	Franz Schrader
1934-1936.	Charles Zeleny	J. F. Daniel	J. S. Nicholas
1935-1937.	D. H. Tennent	L. C. Dunn	C. G. Hartman
1936-1938.	Sally Hughes-Schrader	E. E. Just	D. H. Wenrich
1937-1939.	Leigh Hoadley	W. A. Kepner	J. Percy Moore
1938-1940.	J. H. Bodine	N. E. McIndoo	H. H. Plough
1939-1941.	J. E. Kindred	H. W. Rand	S. A. Matthews
1940-1942.	A. M. Banta	V. Hamburger	C. W. Metz

LIST OF PLACES OF MEETING

AMERICAN MORPHOLOGICAL SOCIETY

1890—Boston	1894—Baltimore	1899—New Haven
1891—Philadelphia	1895—Philadelphia	1900—Baltimore
1892—Princeton	1896—Boston	1901—Chicago
1893—New Haven	1897—Ithaca	1902—Washington
	1898—New York	

CENTRAL NATURALISTS

1899—Chicago	1900—Chicago
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SOCIETY OF AMERICAN ZOOLOGISTS

1901—Chicago	1902—Washington
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AMERICAN SOCIETY OF ZOOLOGISTS

EASTERN BRANCH	JOINT MEETINGS	CENTRAL BRANCH
1903—Philadelphia	1905—Ann Arbor	1903—St. Louis
1904—Philadelphia	1908—Baltimore	1905—(Mch.) Chicago
1906—New York	1911—Princeton	1907—(Mch.) Madison
1907—New Haven	1912—Cleveland	1907—Chicago
1909—Boston	1913—Philadelphia	1910—(Apr.) Iowa City
1910—Ithaca		1910—Minneapolis
		1912—(Apr.) Urbana

MEETING PLACES

1914—Philadelphia	1924—Washington	1934—Pittsburgh
1915—Columbus	1925—New Haven	1935—Princeton
1916—New York	1926—Philadelphia	1936—Atlantic City
1917—Minneapolis	1927—Nashville	1937—Indianapolis
1918—Baltimore	1928—New York	1938—Richmond
1919—St. Louis	1929—Des Moines	1939—Columbus
1920—Chicago	1930—Cleveland	1940—Philadelphia
1921—Toronto	1931—New Orleans	1941—Dallas
1922—Boston	1932—Atlantic City	1942—New York
1923—Cincinnati	1933—Boston	(cancelled)

AMERICAN SOCIETY OF ZOOLOGISTS

CONSTITUTION

ARTICLE I

NAME AND OBJECT

Section 1. The Society shall be called the "American Society of Zoologists."

Sec. 2. The object of the Society shall be the association of workers in the field of zoology for the presentation and discussion of new or important facts and problems in that science and for the adoption of such measures as shall tend to the advancement of zoological science.

ARTICLE II

MEMBERSHIP

Section 1. The membership of the Society shall consist of two classes:

a. Associate membership open to all actively engaged in the field of zoology who have had training in zoology equivalent to that required for the doctorate degree.

b. Active membership open to those who hold the doctor's degree in zoology or who have done work equivalent thereto, and who, in addition to the usual requirements for this degree, have published the results of substantial zoological research.

Sec. 2. Election to membership in the Society shall be upon recommendation of the Executive Committee.

Sec. 3. Associate members shall not hold office or vote, but may present papers before the Society without introduction. Associate members shall receive all literature issued by the Society, including the proceedings of the Society, and shall have the same privileges as active members in journal subscriptions.

Sec. 4. Associate members may be elected to active membership in the usual way when the research requirements of active membership have been met.

ARTICLE III

OFFICERS

Section 1. The officers of the Society shall be a President, a Vice-President, a Secretary, a Treasurer, and the members at large of the Executive Committee.

Sec. 2. The Executive Committee shall consist of the President, the Vice-President, the Secretary, the Treasurer, and five members elected from the Society at large. Of these five members, one shall be elected each year to serve five years. If any member at large shall be elected to any other office, a member at large shall be elected at once to serve the remainder of his term.

Sec. 3. These officers shall be elected by ballot at the annual meeting of the Society and their official terms shall commence with the close of the annual meeting, except that the Secretary and the Treasurer shall be elected triennially and shall serve for three years.

Sec. 4. The officers named in Section 1 shall discharge the duties usually assigned to their respective offices. (See by-laws.)

Sec. 5. Vacancies in the board of officers, occurring from any cause, may be filled by election by ballot at any meeting of the Society. A vacancy in either the Secretaryship or the Treasurership occurring in the interval of the meetings of the Society may be filled, until the next annual meeting, by appointment by the Executive Committee.

Sec. 6. At the annual meeting the President shall name a nominating committee consisting of three active members. It shall be the duty of this committee to nominate officers of the Society, including the permanent representatives which the Society is asked to name to various boards and councils. This committee shall make its nominations to the Secretary at least four months before the next annual meeting. Additional nominations for any office may be made in writing to the Secretary by any five members at any time previous to balloting.

ARTICLE IV

MEETINGS OF THE SOCIETY

Section 1. Unless previously determined by the Society, the time and place of the annual meeting shall be determined by the Executive Committee. Special meetings may be called and arranged for by the Executive Committee. Notices of such meetings shall be mailed to all members of the Society at least two weeks before the date set for the meeting.

Sec. 2. Sections of the Society may be organized in any locality by not less than ten members, for the purpose of holding meetings for the presentation of scientific papers. Such sections shall have the right to elect their own officers and also associate members; provided, however, that associate membership in any section shall not confer membership in the Society.

ARTICLE V

QUORUM

Twenty-five members shall constitute a quorum of the Society and four a quorum of the Executive Committee.

ARTICLE VI

CHANGES IN THE CONSTITUTION

Amendments to this Constitution may be adopted at any meeting of the Society upon approval of two-thirds of the members voting, subject to the following conditions:

a. The proposed amendment must be in writing and signed by at least five members of the Society.

b. This signed proposal must be in the hands of the Secretary at least one month before the meeting of the Society at which it is to be considered.

c. The Secretary shall mail copies of the proposed amendment to the members of the Society at least two weeks before the meeting.

d. Members unable to attend may submit their votes in writing to the Secretary.

BY-LAWS

ARTICLE I

DUES

Section 1. The annual dues for active and associate members, unless remitted or changed by the vote of the Society, shall be two dollars.

Any member in good standing, having paid annual dues to the Society for not less than twenty-five years, who shall change his professional status, may apply to the Executive Committee for the remission of further dues. Applicants approved by the Executive Committee shall be exempt from further payment of dues, but such members shall be entitled to all of the privileges of membership formerly enjoyed.

Sec. 2. Any member in arrears of dues for two years should be dropped by the Executive Committee. Upon payment of arrearage he shall be reinstated.

ARTICLE II

PRESIDENT

Section 1. The President shall preside at scientific sessions and at the business meetings.

Sec. 2. He shall also appoint the committees prescribed by the constitution and by-laws.

ARTICLE III

VICE-PRESIDENT

Section 1. The Vice-President shall preside at sessions designated by the President.

Sec. 2. He shall also assume the duties of the President in the latter's absence.

ARTICLE IV

SECRETARY

Section 1. The Secretary shall keep the records of the Society.

Sec. 2. He shall be responsible for all arrangements for the meetings, including the appointment of local committees.

Sec. 3. Whenever the proper officers of a number of related societies shall have a conference covering matters of mutual interest to the several societies, he shall act as the delegate or representative of this Society.

Sec. 4. He shall make such purchases and employ such assistance as will, in his judgment, expedite the business of the Society, and

Sec. 5. He shall be reimbursed out of the funds of the Society for expenses incurred in attending meetings of the Society.

ARTICLE V

TREASURER

Section 1. The Treasurer shall be in charge of the funds of the Society.

Sec. 2. At the annual business meeting of the Society he shall present a statement to date of the funds of the Society.

Sec. 3. He shall make such purchases and employ such assistance as will, in his judgment, expedite the business of the Society.

ARTICLE VI

AUDITING COMMITTEE

The President shall annually appoint an auditing committee of two, who shall audit and report upon the financial record and statement of the Treasurer at the meeting for which they were appointed.

ARTICLE VII

PROGRAM RULES

In matters relating to programs for annual meetings, the following rules shall be observed:

Section 1. Titles and abstracts of papers to be presented by any method at the annual meeting must be sent to the Secretary in duplicate before the final date set by him. The length, form, and arrangement of the abstracts must comply with the requests of the Secretary in order to insure prompt publication by The Wistar Institute. The titles and abstracts of papers which do not conform to such requests or papers which are received late may be presented by title only.

Sec. 2. Titles shall be listed in groups according to subject matter, the groups to be determined by the Secretary.

Sec. 3. Whenever conditions require, the Secretary shall schedule two or more groups for the same hour and arrange the program to bring together papers on subjects of more general interest for meetings of the whole Society.

Sec. 4. Each member may have not more than fifteen minutes of the time of the Society at the annual meeting. This time may be used by him to present one or more papers personally or to introduce papers of non-members.

Sec. 5. All papers not read when called for as listed shall be placed at the end of the group list, and if not read when called for the second time, shall be read by title only.

Sec. 6. The titles of 'introduced' papers may be listed in the groups after the titles of papers to be read by members.

PROCEEDINGS OF THE AMERICAN SOCIETY OF ZOOLOGISTS

ANNUAL MEETING

The fortieth annual meeting of the American Society of Zoologists, which was scheduled to be held in New York City in conjunction with Section F of the American Association for the Advancement of Science, was postponed on request of The Office of Defense Transportation of the Federal Government. A letter received on November 30th from Dr. F. R. Moulton, permanent Secretary of the American Association for the Advancement of Science, announced the postponement of the Association meeting scheduled for the week of December 28, 1942, in compliance with the above request.

It therefore seemed entirely proper, as well as necessary, for the American Society of Zoologists to do likewise and accordingly the Executive Committee, by mail ballot, voted unanimously to postpone the meeting of the Society scheduled to be held at Hunter College of the City of New York, on December 29, 30 and 31, 1942. The postponement of a meeting generally implies that it will be held at a later date, however, since it does not seem likely because of restrictions on travel, etc., that a regular meeting of the Society can be held during the war period, the Executive Committee has since voted unanimously to cancel the meeting.

The program and abstracts prepared for the New York Meeting were printed and practically ready for distribution when the request for postponement was received. There seemed to be no object in withholding any part of it and accordingly this material was distributed to members of the Society as usual.

Judging from the program and the numerous inquiries directed to the Secretary's Office the New York Meeting gave every promise of success. Most of those closely associated with arrangements confidently felt that had the meeting been

held it would have compared favorably with those of recent years. This is somewhat surprising in view of prevailing conditions but since the meeting was so close at hand and arrangements practically complete when postponement was announced, it is likely that this prediction was essentially correct. The total number of papers on the program scheduled to be presented in person was 121, of which number 17 were offered for demonstration. In addition there were included in the program 95 papers as read "by title." The foregoing figures also include the papers comprising the various symposia that were scheduled.

Before the meetings were postponed, Dr. W. C. Allee, Vice-President of the American Association for the Advancement of Science and Chairman of Section F, had prepared the address normally given at the Annual Dinner of the American Society of Zoologists. This will be published in *Science* (1943) under the title, "Where angels fear to tread; a contribution from general sociology to human ethics."

BUSINESS TRANSACTIONS

The referendum conducted in January on the question of granting the Executive Committee authority to transact the routine business of the Society during the interim showed a response from more than 75% of the members who almost unanimously favored this proposal. Because of the great distances separating the members of the Executive Committee and the restrictions on travel, it was decided to transact the business on the agenda for the fortieth annual meeting by mail. This arrangement may be modified as conditions warrant though it proved to be quite satisfactory. The following business was transacted:

1. *The minutes of the last business meeting.* The minutes of the last meeting were published in the February 1942 supplement to *The Anatomical Record* and distributed to all members of the Society.

The Committee voted unanimously to approve the minutes of the last business meeting as printed.

2. *Memorial resolutions.* The Secretary reported that during the last year the Society had suffered the loss by death of one associate and three active members. These were J. Richard Carpenter, John Franklin Daniel, Robert William Hegner, and Jacob Reighard. The following resolutions were prepared.

J. RICHARD CARPENTER

Dr. J. Richard Carpenter, Professor of Biology in Black Mountain College died in Duke Hospital, Durham, North Carolina, on July 16, 1942. The immediate cause of his death was a thoracic cancer which had been discovered but a few weeks previously in the course of a medical examination incidental to his prospective entry into active service as a First Lieutenant.

Dr. Carpenter was born at Oshkosh, Wisconsin, March 19, 1911 and received his early education in the public schools and Junior College of Grand Rapids, Michigan, where his parents still live. Undergraduate work was completed at the University of Illinois, where his graduate work was begun. He then transferred to the University of Oklahoma where the degrees of M.S. and Ph.D. were granted in 1934 and 1939 respectively. Three years of the interim were spent at Oxford University on a Rhodes Scholarship. At Oxford he worked under the direction of Charles Elton, his thesis being concerned with the periodicity of insect outbreaks in Europe. One vacation period was spent in a trip around the world including a visit of some duration to various scientists and institutions in Soviet Russia. He became familiar with the Russian language and contributed, especially to the *Journal of Animal Ecology*, many reviews of Russian ecological contributions.

While at the University of Oklahoma, he was successively Research Scholar and Graduate Assistant in the Department of Zoology and Assistant in the Biological Survey, and at the time of his death had completed his second year as Professor of Biology at Black Mountain College.

He was an active member of several scientific societies including the Academies of Illinois, Oklahoma, and North Carolina, the American Society of Zoologists, the British Entomological Society, the British Ecological Society and the Ecological Society of America, on whose committee on Nomenclature he served until his death. His publications include one book "An Ecological Glossary" published by the University of Oklahoma Press and in second edition by a London publisher, and thirty or more papers, especially in *Ecological Monographs*, the *Journal of Animal Ecology*, and *Ecology*. While at Oxford, he was a member of the review staff of the *Journal of Animal Ecology* and his abstracts, especially of Russian papers, have appeared regularly in *Biological Abstracts*.

The science of Ecology has lost one of its most promising young investigators, the college with which he was associated has lost an inspiring teacher, and the loss to his friends can be estimated only by those who knew him best.

A. O. WEESE.

JOHN FRANKLIN DANIEL

John Franklin Daniel, son of Dr. John Franklin and Martha Short (Henry) Daniel was born at O'Fallon, Missouri, July 31, 1873 and died at Berkeley, California, November 2, 1942. With his passing the American Society of Zoologists has lost a devoted member, greatly distinguished by a congenial and colorful personality.

It seems that Frank Daniel's interest in nature study was awakened when, as a man of nearly thirty, he found himself placed under the spell of the exuberant tropical nature of the Philippine Islands. He had followed a call of the United States government and served for 4 years ('01-'05) as a teacher and later as a District Superintendent of Schools on the island of Cebu. His first scientific contribution, "Animal Life of Malaysia" ('05) is an immediate outgrowth of this period. As in the case of many other young Americans, the years of service in the Islands shaped much of his later life. The eyes of the midwesterner were opened to the educational values of travel and of new cultural contacts. Frank Daniel became a great traveller, using vacations and sabbatical leaves to gain an ever widening intimacy with the face of this world, its many tongued people and — most interesting to him — its scientists.

On returning from the Philippine Islands he resumed his college education, and received a B.S. degree from The University of Chicago in 1906. He then transferred to The Johns Hopkins University where, under the direction of Prof. H. S. Jennings, he prepared a thesis on the "Adaptation and Immunity of Lower Organisms to Ethyl Alcohol." After a last year of study as an Adam T. Bruce fellow at the Pasteur Institute at Lille, France, he was awarded the Ph.D. degree in 1909.

The following year he spent at the University of Michigan as an Instructor in Zoology and in 1910 accepted a call to the University of California at Berkeley. He served this institution for 32 years, first as an Instructor in Comparative Anatomy and during the last 6 years as Head of the Department of Zoology.

Daniel's post-doctoral research work was almost entirely devoted to the vertebrates. After the transfer to California, he became intensely interested in the anatomy of the sharks. Spending many summer vacations at the Scripps Institution of Oceanography at La Jolla, he published a series of morphological studies which were preliminary to his well known book on "The Elasmobranch Fishes" (University of California Press, '22).

In the early twenties his attention shifted from the sharks to the Californian amphibians. The realization of a well planned program of research in the experimental embryology and endocrinology of newts and tree frogs was greatly promoted by a now rapidly growing staff of students and collaborators. A volume dedicated to the memory of Dr. Daniel is under preparation by the University of California Press which is to contain reports on most recent investigations carried out by his school. This seems a fitting tribute to a man, whose merits were honored by the governments of the United States, through the appointment as delegate to the International Congress of Zoology at Lisbon, and of France, by bestowing the decoration of Chevalier de la Légion d'Honneur.

In 1909 Dr. Daniel married Miss Menetta White Brooks, daughter of William Keith Brooks, and in her he found an understanding wife and companion, always intent on aiding his scientific pursuits.

A man of culture and sympathetic character, he deeply deplored the outbreak of brutality which preceded the present war. Though he must have been aware of the impossibility of stemming the tide, he helped unhesitatingly and mag-

nanimously when our country was sought for shelter by displaced foreign scholars of distinction. As time passes, friends and associates will probably remember Frank Daniel foremost as an amiable representative of the more humane and hopeful age that reigned in the decades before the first world war.

EMIL WITSCHI.

ROBERT WILLIAM HEGNER

Zoology in general and protozoology in particular suffered a severe loss with the passing of Robert William Hegner, the energetic and forceful exponent of medical zoology at The Johns Hopkins University for nearly a quarter of a century.

Dr. Hegner was born in Decorah, Iowa, on February 15, 1880. He graduated from The University of Chicago with the B.S. degree in 1903 and continued there as a graduate student until he transferred to the University of Wisconsin, where he received the Ph.D. degree in 1908. After serving as Instructor and as Assistant Professor of Zoology at the University of Michigan, in 1918 he was called to the School of Hygiene and Public Health of The Johns Hopkins University. Here he began his definitive career, becoming Associate Professor in charge of the Department of Medical Zoology in 1920 and Professor of Protozoology in 1922, a position he held with distinction until the end of his life, March 11, 1942.

The career of Dr. Hegner was one of marked versatility and activity. His early investigations on insect embryology led to the publication of his well-known treatise on "The Germ Cell Cycle of Animals." Thereafter his research turned to the field of protozoology and resulted in the extensive series of papers that flowed from his laboratory. Many were on the general biological aspects of the Protozoa, including genetics, but the majority involved studies on parasitic forms of particular interest in problems of medicine and human welfare.

Coincident with his intensive research program and the direction of the research of nearly fifty graduate students, Dr. Hegner found time to publish a considerable number of books, including technical treatises, textbooks, and popular expositions of zoology. Among them, his "College Zoology," now in its fifth edition, has had an important influence on zoological courses in American colleges.

Dr. Hegner traveled extensively. He was a delegate to the Royal Institute of Public Health at Brussels, Belgium, in 1920, and to the International Congress on Health Problems in Tropical America at Jamaica in 1924. In 1926 he served as exchange Professor at the London School of Hygiene and Tropical Medicine, and later organized a program in protozoology at the School of Hygiene of the University of the Philippines and at the Institute of Public Health in Mexico City. He also directed several expeditions for the study of parasitic Protozoa in tropical American countries.

Dr. Hegner served as President of the American Society of Zoologists and of the American Society of Parasitologists, and Vice-President of the Zoological Section of the American Association for the Advancement of Science. He was one of the founders of the American Academy of Tropical Medicine and a member of the scientific board of The Gorgas Memorial Institute. His scientific contributions were also recognized abroad: he was a fellow of the Royal Society of Tropical Medicine and of the Royal Institute of Public Health, and an honorary member of the Sociedad Mexicana de Historia Natural.

L. L. WOODRUFF.

JACOB REIGHARD

It was Jacob Reighard's first stroke of luck to have been saved by the blissful self-concern of infancy from the horrors of a nation torn asunder by civil strife; it was his last good fortune to have been spared by death from the calamity that now befalls the whole world: he was born on July 2, 1861, in La Porte, Indiana, and died on February 13, 1942, in Ann Arbor, Michigan.

Reighard lived through the rise of modern zoology and contributed eminently to the development of his chosen science. For more than 40 years, after having been trained at Michigan and under Mark at Harvard, he built and guided the several zoological units at the University of Michigan. The Department of Zoology, the Museum of Zoology, the Biological Station, the Research Club, the Michigan Academy of Science, and the University Club, are living monuments to his abilities in organizing and in developing. The Great Lakes laboratory of the Bureau of Fisheries (now the Fish and Wildlife Service) is a direct outgrowth, and the Institute for Fisheries Research of the Michigan Department of Conservation an indirect product, of his work for the federal and state fishery agencies. In 1903 he served as the first President of the Central Branch of the American Society of Zoologists, and as the first Vice-President of the Eastern Branch.

Scores of zoologists, including many leaders, were trained by Reighard in zoology and in rigorous scientific discipline. Many others profitted from and enjoyed their contacts with him.

Reighard's main published contributions were on the anatomy of the cat, a well-known teaching manual written with Herbert S. Jennings as junior author; on the embryology of fishes, as related to problems of morphology and of fish culture; on the methods of fish culture and of fisheries surveys; on the limnology of the Great Lakes, with particular reference to the plankton and to the fisheries; on the biological significance of the bright colors of coral-reef fishes (Reighard's researches at Tortugas — models of experiments in nature — led him to explain the facts by a theory of immunity coloration as an alternative to one of warning coloration); on the courting and nesting habits of fishes, and on the methods of studying these breeding habits, especially by photography (it was particularly in these researches that Reighard displayed his unusually keen powers of observation and analysis); finally on the theory and practice of lip-reading, into which subject Reighard was lead by his own growing affliction of deafness.

Professor Reighard was a master and a pioneer in both pure and applied zoology. Each of the series of investigations to which he successively devoted himself was prosecuted with extreme thoroughness and completeness; with constant attention to detail, yet with the main objective in mind; with unfaltering logic; with originality and foresight.

CARL L. HUBBS.

The foregoing resolutions were unanimously adopted by the Committee and the Secretary was instructed to send copies to the nearest relatives and to publish the resolutions in the Proceedings of the Society.

3. *Report of the Treasurer.* The Treasurer presented the following report for the year 1942:

Credits:

Cash balance from 1941	\$1201.27	
U. S. \$1000 Savings Bonds M305107D and M305109D; present redemption value at \$800	1600.00	
Receipts from dues	3444.50	
Total		\$6245.77

Expenditures:

Miscellaneous Expenses, Dallas	52.98	\$ - 52.98
Secretary's Expenses:		
Stenographer and steno. supplies	214.74	
Printing, stationary, postage	162.23	
Travel	78.62	
Stipend	200.00	
Cable, telephone and telegraph	13.54	669.13

Treasurer's Expenses:

Stenographer	17.64	
Postage, stationary	44.31	
Printing	59.31	121.26

The Wistar Institute:

Programs and abstracts	716.99	
Proceedings	277.56	994.55

Biological Abstracts:

Special contribution through Treasurer...	2.00	
Subscriptions through Treasurer	3.00	
Contributions from members	1605.50	1610.50

Bank charges		4.35
Members' checks returned		12.00
Total expenditures		\$3464.77

Balance, December 31, 1942

Checking account	1181.00	
Two U. S. Savings Bonds at \$800	1600.00	2781.00
Total		6245.77
Total December 23, 1941		5878.89
Increase of funds handled by Treasurer dur- ing current year		366.88
Decrease in assets of the Society during cur- rent year		20.27

4. *Report of the Auditing Committee.* The Auditing Committee consisting of F. B. Adamstone and L. J. Thomas presented the following report:

We have examined the books of the American Society of Zoologists and find that the records of receipts and expenditures as shown in the day book, check book, cancelled checks, bank book and bank statements of receipts and expenditures by H. W. Beams for the year 1942, are in good order.

The amounts stated in the annual report of the Treasurer are also correct in that they show an increase in the amount of funds handled during the current year but net decrease in the total assets of the Society.

The Committee voted to accept and approve the reports of the Treasurer and of the Auditing Committee.

5. *Remission of dues.* The Treasurer makes the following recommendation in order to bring before the Executive Committee the question of dues as it concerns foreign members and members in the armed services:

It is suggested that payment of dues by members in the armed services and by foreign members be suspended for the duration in order that the embarrassment of being dropped for non-payment of dues be avoided, i.e., no member should be dropped for non-payment of dues for the duration.

Since it is impossible for the Treasurer to know who is not in service it is suggested that the following statement be enclosed in the notices of dues to all members:

Members in the armed forces and foreign members are exempted from payment of dues for the duration.

The Committee voted unanimous approval of this recommendation.

6. *Support of Biological Abstracts.*

Comprehensive reports submitted by the Editor-in-Chief and Business Manager of Biological Abstracts indicate a continued need for substantial support from Biological Societies if present standards are to be maintained. It was therefore recommended that the American Society of Zoologists provide the same support in 1943 as for 1942, namely, that \$2.00 from each membership fee of \$4.00 be paid to Biological Abstracts.

The Committee upon careful examination of the above reports voted to approve this recommendation.

7. *Election of new members.* In accordance with the stipulations in the constitution regarding membership the following candidates were recommended for election to membership in the Society.

ACTIVE MEMBERS

(Formerly Associate Members)

Finley, Harold Eugene	Hasler, Arthur Davis	Phillips, Frederick Stanley
Forster, Roy Philip	Mickey, George Henry	Reinhard, Edward George
Gorbman, Aubrey	Miller, John Allen	Spratt, Nelson Tracy, Jr.
Hahnert, William Franklin	Old, Marcus Calvin	Wharton, George Willard

(Not Formerly Associate Members)

Burkenroad, Martin David	Gilbert, Chauncey McLean
Caspari, Ernst Wolfgang	Griffen, Allen Beattie
Chen, Tze-Tuan	Hall, E(ugene) Raymond
Fennell, Richard Adams	Liebmann, Emil
Foerster, Russell Earle	Steggerda, Morris
Fries, Erik Fritiof Bjerkander	

NEW ASSOCIATE MEMBERS

Burbank, William Dudley	Johnson, J. Garth	Smith, Charles Clinton
Dropkin, Victor H.	Kent, George Cantine, Jr.	Smith, Frederick
Edmondson, W(alles) Thomas	Lowrie, Donald C.	George Walton
Forbes, Grace Springer	Miller, Elwood Morton	Talbot, Mary
Gilbert, Perry Webster	Moody, Paul Amos	Todd, Arlie C.
Gregg, Robert E.	O'Brien, John Patrick	Villee, Claude Alvin, Jr.
Harman, Pinckney Jones	Ryan, Francis Joseph	Walker, Roland
Hewitt, William Francis, Jr.	Sanford, Katherine Koontz	Wilbur, Charles Grady
		Wulff, Verner John

The Committee voted that all the above named candidates be declared elected to the classes of membership indicated and instructed the Secretary to cast one ballot in their favor.

8. Changes in membership. For the information of the Society the Secretary reports the following changes in membership which have occurred during the past year. The following summary includes the above newly elected candidates:

Deaths	4	New associate members	24
Resignations	3	Reinstated	1
Dropped	0	New active members	10
	—		—
	7		35

Total enrollment in last report	1001
Gain in enrollment	28
	—
Total enrollment at present	1029

9. *Election of officers.* The Nominating Committee consisting of Fernandus Payne, Chairman; Victor C. Twitty; and David H. Wenrich, presented the following report:

President, 1 year: T. S. Painter.

Vice-President, 1 year: L. H. Snyder.

Secretary, 3 years: L. V. Domm.

Executive Committeeman, 5 years: L. L. Woodruff.

Society Representatives on Council of A.A.A.S., 1 year: H. H. Plough; T. M. Sonneborn.

Society Representatives on Council of Union of American Biological Societies, 1 year: J. H. Bodine; J. P. Visscher.

Society Representatives on Advisory Editorial Board of Biological Abstracts: L. V. Heilbrunn; A. I. Ortenburger; A. Franklin Shull; H. J. Van Cleave.

Society Representative on the Executive Committee of the Commission for the Standardization of Biological Stains: S. I. Kornhauser.

Members of Editorial Board of the Journal of Morphology, 3 years: L. B. Arey; A. B. Dawson; C. C. Speidel.

The Committee unanimously voted that the above-named candidates be elected and the Secretary was instructed to cast one ballot in their favor.

10. *Committee Reports.*

(a) The following report by S. I. Kornhauser, Representative of the Society on the Executive Committee of the Commission for the Standardization of Biological Stains, was presented:

I wish to report to the Society, the activities of the Commission on the Standardization of Biological Stains and my work as representative of the American Society of Zoologists.

The demand for certified stains has been greater than ever anticipated by the Commission. It is probable that the increased requirements of the armed forces and the public health establishments have caused this rise. This demand has required the testing of numerous batches of dyes. The stains are meeting the standards of the Commission; and the biological and medical requirements of this country and our allies are being adequately met.

When we compare our favorable position at this time with the lack of adequate stains during the first World War and the years following we can feel some sense of satisfaction and give praise to those who had the foresight to establish the Commission especially to Dr. H. J. Conn who has been the guiding spirit of the enterprise since its inception.

The increased revenue from certification labels has been sufficient not only to pay all current expenses but also to set aside funds for the future and for research on stains.

One notable achievement of the past year has been the production of synthetic orcein for elastic tissue by MacAndrews and Forbes. Orcein was no longer obtainable owing to the impossibility of importing the vegetable material from which it was manufactured. Your representative was especially interested in orcein in connection with the development of a quadruple tissue stain and aided in testing and comparing the experimental orceins. I am glad to state that the synthetic product is superior to most of the natural orceins previously used by me.

Stain Technology has fared well and the increase in domestic subscriptions has counterbalanced the previous loss of foreign subscriptions. Your representative has aided in the editing of numerous papers submitted for publication.

The Commission is now in the process of putting out a loose leaf manual of staining methods.

Your representative has continued as treasurer of the Commission and has done considerable testing of such stains as azocarmine, tetrachrome blood stain, Nile blue sulphate, Congo red and carmine.

(b) The War Emergency Subcommittee of the Executive Committee consisting of C. G. Hartman, Chairman; M. H. Jacobs, J. S. Nicholas, and L. L. Woodruff and L. V. Domm, Ex officio, presented the following report:

Early in 1942 President Woodruff appointed Messrs. Coker (Chairman), Jacobs, Hartman and Domm members of a War Emergency Subcommittee of the Executive Committee of the Society. After some correspondence with the committee members in which he made numerous useful suggestions, Chairman Coker found it necessary to resign because of many other emergency duties. The present chairman was asked to take over the chairmanship and Doctor Nicholas, already a member of a similar committee of the American Association of Anatomists, accepted membership vice Doctor Coker.

Every effort was made to ascertain the views of the heads of the armed services in Washington as to how zoologists could make the best contributions to the war effort. Two circular letters reporting progress were prepared and sent to heads of departments, one in August, the second early in December.

For the most part, all the advice we could get was for zoologists to continue in their teaching and research, enroll with the Roster and await orders.

Not satisfied with this, our representatives on the National Research Council, the Division of Biology and Agriculture under Dr. Robert F. Griggs, seconded by President Ross G. Harrison, have put forth every effort to make the authorities "biology conscious," emphasizing the growing need for food and other products of plant and animal origin. To further this end the National Research Council in August called a meeting of biologists to discuss the problem (see Bulletins of the Division of Biology and Agriculture). The recent efforts of the Man Power Board to save the situation are doubtless largely due to the efforts of the National Research Council, ably assisted by biologists throughout the country, including those responding to the circular letters of this committee. These letters were not usually answered but were carefully studied and found exceedingly helpful.

The problem still remains, however, to find places for our zoologically trained men and women to serve in a manner most useful to the war effort. Enrollment with the National Roster of Scientific and Specialized Personnel, U and 10th Streets, Washington, D. C., is still in order.

Any new development of special interest to zoologists will be communicated to the membership of the Society.

(c) The Motion Picture Committee, consisting of Ralph Buchsbaum, Robert Chambers, Jr., and O. W. Richards, working with E. J. Farris, representative of The Wistar Institute, submitted the following report:

The Motion Picture Committee of the American Society of Zoologists, along with a similar committee representing the American Association of Anatomists, has continued its cooperation during the past year with The Wistar Institute in evaluating and reviewing films in biology. The committee has selected The Anatomical Record for the listing of approved films in zoology and those approved during 1942 were published in this Journal.

An American Committee on Biological and Medical Films was created during 1942 for the purpose of coordinating scientific groups interested in planning, producing, and using biological and educational films. The American Society of Zoologists will be invited to be represented when this group meets.

The Committee voted to approve the foregoing reports.

11. Appointment of an Advisor on Cycles. The following memorandum from Dr. Ellsworth Huntington, of the Foundation for the Study of Cycles, New York City, outlines a project for which he solicits the cooperation of the American Society of Zoologists to the extent of appointing an Advisor whose duty it shall be to keep the foundation informed on publications in zoology pertaining to cycles and to give advice respecting publications on the subject worthy of consideration.

The Foundation for the Study of Cycles—a non-profit organization created to foster, promote, and conduct scientific research in respect to rhythmic and periodic fluctuations in any branch of science—announces the offering of a medal to the person who, during 1943, published the book or paper that in the opinion of the judges is the most outstanding. It is hoped that the announcement of these awards in journals of various kinds will help to make people realize how widely the study of cycles enters into diverse sciences, such as astronomy, botany, economics, geography, psychology, zoology, and so on.

The judges who will award the medal are Dr. C. G. Abbot, Solar Physicist and Secretary of the Smithsonian Institution; Dr. Harold E. Anthony, Curator of

Mammals and Dean of Scientific Staff, American Museum of Natural History; Prof. W. C. Mitchell, Professor of Economics, Columbia University, and Director of the National Bureau of Economic Research; Prof. V. C. Wynne-Edwards, Professor of Zoology, McGill University; and Prof. Ellsworth Huntington, Professor of Geography and Climatology, Yale University (Chairman).

Part of the proposed plan is to ask scientific societies to appoint advisors to cooperate with the Foundation for the Study of Cycles. We shall ask the advisors to send us copies of all articles pertaining to cycles in their fields, or at least to give us references. This will help in building up a reference library and card catalog for general use at the office of the Foundation in New York. We shall also ask each advisor to indicate his opinion as to the most valuable contributions of the year to cyclic studies in his particular field. The medallist and the receivers of honorable mention will be chosen from the selections thus made.

The Foundation urges caution regarding the confusion of contributions to our knowledge of interrelationships with contributions to the knowledge of rhythms and periodicities per se. There may be an excellent correlation between two phenomena, and yet no rhythms may be involved in either. The direct concern of the Foundation for the Study of Cycles is only with rhythms and periodicities as such. Denial of the existence of a rhythm may be as important as affirmation.

Communications should be addressed to Prof. Ellsworth Huntington, Chairman of the Committee on Awards, Hendrie Hall, Yale University, New Haven, Connecticut.

The Committee voted to approve the foregoing request and instructed the President to appoint an advisor to cooperate with the Foundation for the Study of Cycles.

12. Cancellation of the New York Meeting. The program which was distributed to members of the Society in December announced the postponement of this meeting. Since postponement of a meeting generally implies that it will be held at a later time and since it does not seem likely, because of restrictions imposed by war conditions, that a regular meeting can be held at this time, it was considered advisable to take official action in the matter.

The Committee upon due consideration voted unanimously to cancel the 1942 meeting and instructed the Secretary to publish an appropriate statement in the Proceedings.

13. Nominating Committee for 1943. The President appointed the following nominating committee to prepare a slate of officers for 1943: C. E. McClung, Chairman; Mrs. E. B. Harvey, and T. M. Sonneborn.

14. Vote of appreciation. The local committee consisting of Bernice L. Maclean, Chairman; P. L. Bailey, Jr., L. C. Dunn, Herbert Elftman, James Forbes, Florence Lowther, Theodora Nelson, Mervin Oakes, Priscilla Pollister, Roberts Rugh, and Carl J. Sandstrom, had virtually completed arrangements for the New York Meeting when the announcement of its postponement was received.

The Executive Committee voted unanimously to express the appreciation of the Society to the local committee and to Hunter College of the City of New York for their untiring efforts to make the meeting a success.

L. V. DOMM,
Secretary.

LIFE AND ACTIVE MEMBERS

(* indicates life members of the Society; † indicates charter members of the American Morphological Society; mail addresses are in italics.)

- ABRAMOWITZ, ALEXANDER A., B.A. (St. Stephen's), M.A., Ph.D. (Harvard), Research Assistant, *Biological Laboratories, Harvard University, Cambridge, Mass.*
- ACKERT, JAMES EDWARD, A.B., A.M., Ph.D. (Illinois), Professor of Zoology and Parasitologist, Agricultural Experiment Station; Dean, Graduate School; *Kansas State College, Manhattan, Kan.*
- ADAMS, A. ELIZABETH, B.A. (Mt. Holyoke), M.A. (Columbia), Ph.D. (Yale), Professor of Zoology, *Mount Holyoke College, South Hadley, Mass.*
- ADAMS, LEVERETT ALLEN, A.B., A.M. (Kansas), Ph.D. (Columbia), Professor of Zoology, Curator of the Museum of Natural History, University of Illinois, *401 Vermont St., Urbana, Ill.*
- ADAMSTONE, FRANK BOLTON, B.A., M.A., Ph.D. (Toronto), Associate Professor of Zoology, *345 Natural History Building, University of Illinois, Urbana, Ill.*
- ADELMANN, HOWARD BERNHARDT, A.B., A.M., Ph.D. (Cornell), Professor of Histology and Embryology, *Department of Zoology, Cornell University, Ithaca, N. Y.*
- ADOLPH, EDWARD FREDERICK, A.B., Ph.D. (Harvard), Associate Professor of Physiology, School of Medicine, *University of Rochester, Rochester, N. Y.*
- ALEXANDER, (EDWARD) GORDON, A.B. (Central), A.M., Ph.D. (Princeton), Professor of Biology, *University of Colorado, Boulder, Colo.*
- ALLEE, WARDER CLYDE, S.B., LL.D. (Earlham), S.M., Ph.D. (Chicago), Professor of Zoology, *Hull Zoological Laboratory, The University of Chicago, Chicago, Ill.*
- ALLEN, BENNET, Ph.B. (De Pauw), Ph.D. (Chicago), Professor of Zoology, University of California, Los Angeles, *909 Micheltorena St., Los Angeles, Calif.*
- ALLEN, EDGAR, A.M., Ph.D., Sc.D. (Brown), Professor of Anatomy, Yale University, *333 Cedar St., New Haven, Conn.*
- ALLEN, WILLIAM RAY, A.B., A.M., Ph.D. (Indiana), Professor of Zoology, *Department of Zoology, University of Kentucky, Lexington, Ky.*
- ALTENBURG, EDGAR, A.M., Ph.D. (Columbia), Assistant Professor of Biology, *Rice Institute, Houston, Texas.*
- ANDERSON, BERTIL G., A.B. (Augustana), M.S., Ph.D. (Iowa), Research Associate, *Department of Botany and Zoology, West Virginia University, Morgantown, W. Va.*
- †*ANDREWS, ETHAN ALLEN, Ph.B. (Yale), Ph.D. (Johns Hopkins), Professor of Zoology, Emeritus, *The Johns Hopkins University, Baltimore, Md.*
- ANGERER, CLIFFORD A., A.B. (Columbia), Ph.D. (Pennsylvania), Instructor in Physiology, *Department of Physiology, Ohio State University, Columbus, Ohio.*

- AREY, LESLIE BRAINERD, Ph.D. (Harvard), Sc.D. (Colby), Robert L. Rea Professor of Anatomy and Chairman of the Division of Anatomy, *Northwestern University Medical School, 303 E. Chicago Ave., Chicago, Ill.*
- ARMSTRONG, PHILIP B., B.S. (Mass. State), M.D. (Cornell), Professor of Anatomy, *Department of Anatomy, College of Medicine, Syracuse University, Syracuse, N. Y.*
- BAGG, HALSEY JOSEPH, B.S., A.M., Ph.D. (Columbia), Research Fellow in Biology, Huntington Fund for Cancer Research, Cornell University Medical College, *Memorial Hospital, 68th St. and York Ave., New York, N. Y.*
- BAITSELL, GEORGE ALFRED, B.S. (Central College, Iowa), M.A., Ph.D. (Yale), Professor of Biology, *Osborn Zoological Laboratory, Yale University, New Haven, Conn.*
- BAILEY, PERCY LAURANCE, JR., A.B., Ph.D. (Brown), Assistant Professor of Biology, *College of the City of New York, Convent Ave. and 139th St., New York, N. Y.*
- BAKER, HORACE BURRINGTON, B.S., Ph.D. (Michigan), Professor of Zoology, University of Pennsylvania, *Zoological Laboratory, University of Pennsylvania, Philadelphia, Pa.*
- BALL, GORDON HAROLD, B.S., M.S. (Pittsburgh), Ph.D. (California), Associate Professor of Zoology, *University of California, Los Angeles, Calif.*
- BALL, STANLEY CRITTENDEN, Ph.B., Ph.D. (Yale), Associate Professor of Biology and Curator of the Zoological Collection, *Peabody Museum, Yale University, New Haven, Conn.*
- BANGHAM, RALPH VANDERVORT, A.B. (Wilmington), B.S., A.M. (Haverford), Ph.D. (Ohio State), Professor of Biology and Head of the Department, *Wooster College, Wooster, Ohio.*
- BANTA, ARTHUR MANGUN, A.B., A.M. (Indiana), Ph.D., (Harvard), Research Professor of Biology, Brown University, *Biology Department, Brown University, Providence, R.I.*
- BARBOUR, THOMAS, A.B., A.M., Ph.D. (Harvard), Sc.D. (Havana; Dartmouth), S.D. (Harvard), Professor of Zoology; Director, Harvard University Museum and *Museum of Comparative Zoology, Cambridge, Mass.*
- BARNES, THOMAS CUNLIFFE, B.A. (Cornell), D.Sc. (Harvard), Professor of Physiology and Pharmacology, *Oglethorpe University Medical School, Oglethorpe University, Ga.*
- BARROWS, WILLIAM MORTON, B.S. (Michigan Agricultural College), S.B., S.M., S.D. (Harvard), *Department of Zoology and Entomology, Ohio State University, Columbus, Ohio.*
- BARTELMIZ, GEORGE WILLIAM, Ph.D. (Chicago), Professor of Anatomy, *Hull Anatomical Laboratory, The University of Chicago, Chicago, Ill.*
- BARTH, LESTER G., A.B. (Wayne University, Detroit), A.M. (Michigan), Ph.D. (Chicago), Assistant Professor of Zoology, *Department of Zoology, Columbia University, New York, N. Y.*
- BARTSCH, PAUL, B.S., M.S., Ph.D. (State University of Iowa), Sc.D. (George Washington), Professor of Zoology, Emeritus, George Washington University; Curator of the Divisions of Mollusks and Cenozoic Invertebrates, *United States National Museum, Washington, D. C.*

- BAUMGARTNER, WILLIAM JACOB, A.B., A.M. (Kansas), Ph.D. (Munich), Professor of Zoology, University of Kansas, 1209 Ohio St., Lawrence, Kan.
- BEADLE, GEORGE WELLS, B.S., M.S. (Nebraska), Ph.D. (Cornell), Professor of Biology (Genetics), Department of Biology, Stanford University, Stanford University, Calif.
- BEAMS, HAROLD WILLIAM, A.B. (Fairmount), M.A. (Northwestern), Ph.D. (Wisconsin), Professor of Zoology, Zoology Department, State University of Iowa, Iowa City, Iowa.
- BEAN, ELIZABETH SMITH, A.B. (Cincinnati), M.A., Ph.D. (Wisconsin), Instructor in Histology and Embryology, Dalhousie University, 28 Chestnut St., Halifax, Nova Scotia.
- BECCARI, NELLO, M.D. (Florence), Professor of Comparative Anatomy, Istituto di Anatomia Comparata, Via Romana, 19, Florence, Italy.
- BECK, LYLE VIBERT, A.B. (Wabash College), M.S. (Washington University), Ph.D. (Pittsburgh), Instructor in Physiology, Hahnemann Medical College, Philadelphia, Pa.
- BECKER, ELERY RONALD, A.B. (Colorado), Sc.D. (Johns Hopkins), Professor of Zoology, Iowa State College, Ames, Iowa.
- BECKWITH, CORA JIPSON, B.S. (Michigan), M.A., Ph.D. (Columbia), Professor of Zoology, Emeritus, Vassar College, Poughkeepsie, N. Y.
- BEERS, CHARLES DALE, A.B., A.M. (North Carolina), Ph.D. (Johns Hopkins), Professor of Zoology, University of North Carolina, 707 Gimghoul Road, Chapel Hill, N. C.
- BEHRE, ELLINOR HELENE, A.B. (Radcliffe), Ph.D. (Chicago), Professor of Zoology, Louisiana State University, Box 8729, University Station, Baton Rouge, La.
- BENNETT, HARRY JACKSON, B.S. (Louisiana), M.S., Ph.D. (Illinois), Associate Professor of Zoology and Assistant to the Dean of the College of Arts and Sciences, Louisiana State University, Baton Rouge, La. (On leave for military service, '42. Capt., U. S. A.)
- BENNITT, RUDOLF, S.B., A.M. (Boston), A.M., Ph.D. (Harvard), Professor of Zoology, University of Missouri, Columbia, Mo.
- BERGER, CHARLES ALBERT, A.B., M.A. (Boston College), Ph.D. (Johns Hopkins), Professor of Cytology, Director of the Biological Laboratory, Fordham University, New York, N. Y.
- BERTHOLF, LLOYD MILLARD, A.B. (Southwestern), A.M., Ph.D. (Johns Hopkins), Dean of the Faculty and Professor of Biology, Western Maryland College, Westminster, Md.
- BEVELANDER, GERRIT, A.B. (Hope), A.M. (Michigan), Ph.D. (Johns Hopkins), Assistant Professor of Anatomy, New York University, College of Dentistry, 477 First Ave., New York, N. Y.
- BIGELOW, HENRY B., A.B., A.M., Ph.D. (Harvard), Sc.D. (Yale), Professor of Zoology, Harvard University; Curator of Oceanography, Museum of Comparative Zoology; Museum of Comparative Zoology, Cambridge, Mass.
- BIGELOW, MAURICE ALPHEUS, B.S., LL.D. (Ohio Wesleyan), M.S. (Northwestern), Ph.D. (Harvard), Sc.D. (Columbia), Professor of Biology, Emeritus, Teachers College, Columbia University, 525 W. 120th St., New York, N. Y.
- BISHOP, SHERMAN C., B.S., Ph.D. (Cornell), Professor of Vertebrate Zoology, University of Rochester, Rochester, N. Y.

- BISSONNETTE, THOMAS HUME, M.A. (Queens), Ph.D. (Chicago), Professor of Biology and Head of the Department, *Trinity College, Hartford, Conn.*
- BLAKE, CHARLES HENRY, S.B., Ph.D. (Massachusetts Institute of Technology), Assistant Professor of Zoology, *Massachusetts Institute of Technology, Cambridge, Mass.*
- BLANDAU, RICHARD JULIUS, A.B. (Linfield), Ph.D. (Brown), Instructor in Biology, *Arnold Biological Laboratory, Brown University, Providence, E. I.*
- BLOOM, WILLIAM, A.B., M.D. (Johns Hopkins), Professor of Anatomy, *Hull Anatomical Laboratory, The University of Chicago, Chicago, Ill.*
- BLOUNT, RAYMOND FRANK, B.S.A., M.S. (Arizona), Ph.D. (Yale), Assistant Professor of Anatomy, *University of Minnesota, Minneapolis, Minn.*
- BLUM, HAROLD FRANCIS, A.B., Ph.D. (California), Principal Biologist (Biophysics), *Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md.*
- BODENSTEIN, DIETRICH, *Department of Zoology, Columbia University, New York, N. Y.*
- BODINE, JOSEPH HALL, A.B., Ph.D. (Pennsylvania), Professor of Zoology, *Zoological Laboratory, State University of Iowa, Iowa City, Iowa.*
- BOELL, EDGAR JOHN, B.A. (Dubuque), Ph.D. (Iowa), Associate Professor of Biology, *Osborn Zoological Laboratory, Yale University, New Haven, Conn.*
- BORING, ALICE MIDDLETON, A.B., A.M., Ph.D. (Bryn Mawr), Professor of Biology, *Yenching University, Yenching University, Peking, China.*
- BOUGHTON, DONALD CLARKE, B.S. (Pittsburgh), M.S. (Minnesota), Ph.D. (Wisconsin), Assistant Professor, *Department of Zoology, University of Georgia, Athens, Ga.*
- BOZLER, EMIL, Ph.D. (Munich), Associate Professor, *Department of Physiology, Hamilton Hall, Ohio State University, Columbus, Ohio.*
- BOYDEN, ALAN ARTHUR, A.B., Ph.D. (Wisconsin), Associate Professor of Zoology, *Rutgers University, New Brunswick, N. J.*
- BOYDEN, EDWARD ALLEN, A.B., A.M., Ph.D. (Harvard), Professor of Anatomy and Chairman of the Department, *University of Minnesota, School of Medicine, Minneapolis, Minn.*
- BRAGG, ARTHUR NORRIS, B.S. (Bates), M.A. (Boston University), Ph.D. (Oklahoma), Assistant Professor of Zoology, *Department of Animal Biology, University of Oklahoma, Norman, Okla.*
- BRAMBEL, CHARLES EUGENE, A.M., Ph.D. (Johns Hopkins), Instructor in Zoology, *The Johns Hopkins University; Biochemist, Mercy Hospital, Baltimore, Md.*
- BRANCH, HAZEL E (LISABETH), A.B., A.M. (Kansas), Ph.D. (Cornell), Professor of Zoology, Head of Department, *University of Wichita, Wichita, Kan.*
- BRAUER, ALFRED, A.B. (Kansas), M.A. (Oklahoma), Ph.D. (Chicago), Professor of Zoology, *University of Kentucky, Lexington, Ky.*
- BREDER, CHARLES MARCUS, JR., D.Sc. (Newark), Director, New York Aquarium, Battery Park, New York, N. Y., and Research Associate, *American Museum of Natural History, New York, N. Y.*
- BRELAND, OSMOND P., B.S. (Mississippi State), Ph.D. (Indiana), Assistant Professor of Zoology, *University of Texas, Austin, Texas.*
- BRENNEMAN, WILLIAM RAYMOND, A.B. (Indiana Central College), Ph.D. (Indiana University), Associate Professor, *Waterman Institute and Zoology Department, Indiana University, Bloomington, Ind.*

- BRINLEY, FLOYD JOHN, B.S. (Colorado State), M.A., Ph.D. (Pennsylvania), Biologist, *U. S. Public Health Service, State Department of Health, Austin, Texas.*
- BROWMAN, LUDVIG GUSTAV, B.S., Ph.D. (Chicago), Associate Professor of Zoology, *Department of Zoology, Montana State University, Missoula, Mont.*
- BROWN, FRANK ARTHUR, JR., A.B. (Bowdoin), A.M., Ph.D. (Harvard), Associate Professor of Zoology, *Department of Zoology, Northwestern University, Evanston, Ill.*
- BROWN, LELAND ARTHUR, S.B. (Denison), A.M. (Pittsburgh), Ph.D. (Harvard), Professor of Biology, *Transylvania College, Lexington, Ky.*
- BROWN, MORDEN GRANT, A.B., A.M., Ph.D. (Stanford), *Spencer Lens Company, Buffalo, N. Y.*
- BRUES, CHARLES THOMAS, B.S., M.S. (Texas), Professor of Entomology, *Biological Laboratories, Harvard University, Cambridge, Mass.*
- BRUNER, HENRY LANE, A.B. (Eureka-Abingdon), Ph.D. (Freiburg), Professor of Zoology, Emeritus, *Butler University, 324 S. Ritter Ave., Indianapolis, Ind.*
- BUCHANAN, JAMES WILLIAM, B.S. (Ohio), Ph.D. (Chicago), Professor of Zoology, *Northwestern University, Evanston, Ill.*
- BUCHSBAUM, RALPH, B.S., Ph.D. (Chicago), Assistant Professor of Zoology in the College, *The University of Chicago, Chicago, Ill.* (On leave for military service, '42. 1st Lt., U. S. A. Air Force.)
- BUCK, JOHN BONNER, A.B., Ph.D. (Johns Hopkins), Assistant Professor of Zoology, *Department of Zoology, University of Rochester, Rochester, N. Y.*
- BUDINGTON, ROBERT ALLYN, B.A., M.A., D.Sc. (Williams), Professor of Zoology, *Oberlin College, Oberlin, Ohio.*
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Totals

Life members	2
Active members	733
Associate members	294
Grand total	1029

A CASE OF HORSESHOE KIDNEY AND ASSOCIATED VASCULAR ANOMALIES IN THE DOMESTIC CAT

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THREE FIGURES

Congenital anomalies of the kidney are known to occur frequently in man. They have been described and classified in considerable detail, and the relative frequency with which various types occur has been established. Comparable data from non-human material are extremely meager, however, even for the well known laboratory animals.

In man one of the common renal anomalies is fusion of the kidneys across the midline to form a so-called "horseshoe" kidney. Since the report of Berengarius (1521) more than 500 cases have been published (Boyden, '31). According to Bell ('41) the incidence is approximately one in 400. Johnson ('14) appears to have been the first to describe fusion of the kidneys in the domestic cat. He records one instance of fusion out of 400 cats dissected in laboratory routine, but his illustration plainly shows a four-lobed, posteriorly fused kidney that bears little resemblance to the horseshoe kidney of human pathology. Ekman ('28) described a "cake-shaped" kidney with crossed dystopia in a domestic cat in great detail. His attempts to explain the origin of this structure and the associated vascular anomalies are marred, however, by his lack of familiarity with the important work of English and American anatomists.

The nearly perfect case of horseshoe kidney described below was discovered during routine laboratory dissection. I am indebted to Dr. H. J. Eigenbrodt, of North Central College,

Naperville, Illinois, for the privilege of describing the specimen F.M.N.H. 1337. Special thanks are also due Mr. D. Dwight Davis, Curator of Anatomy at Field Museum, for his valuable suggestions and criticism. The animal was an adult female. It had been skinned and partially dissected, with a consequent loss of body weight. Fortunately the organs and vessels of the abdominal cavity were left in situ, and hence permitted thorough study. Dr. C. F. W. McClure has kindly consented to my use of an adaptation of his figure of the embryonic veins of the domestic cat.

THE KIDNEYS

The kidneys (figs. 1, 2) lie almost symmetrically across the midline at the normal level, between the third and fifth lumbar vertebrae, ventral to the aorta and right caval vein, but dorsal to the inferior mesenteric artery and left caval vein. The medial borders of the posterior extremities of the kidneys are fused into a single continuous structure that forms a distinct horseshoe. Fusion is more extensive dorsally than ventrally. The anterior extremities remain free. The outline of the original halves is delineated by a constriction at the posterior border of the fused organ. The anterior pole of the right kidney extends 8 mm. farther forward than that of the left, as is usual. There is a simple dystopia of the fused organ toward the right side. The right kidney is oriented normally, with its long axis converging toward the midline anteriorly, where it is actually ventral of the right vena cava, considerably nearer the spinal column than usual. The left organ is shifted medially clear across the aorta, with its posterior extremity ventral of the vena cava, where it meets the corresponding surface of the right kidney. The fused organ at its juncture lies against the vertebral column. The common hilus is located on the ventral surface. A low ridge of the capsule outlines the posterior border of the pear-shaped hilus. The renal pelves are separate and ventrally directed. Both ureters are present, and course ventrad of the united part of the kidneys toward their respective sides of the bladder.

The left ureter is at first dorsad of the left caval vein, gradually shifting to a ventral position. The suprarenal bodies and genitalia are normal.

Longitudinal section (fig. 1) reveals that one continuous fibrous capsule encloses the fused organ, which is composed of typical renal tissue with no evidence of pathological histology. There is an area of cortical parenchyma along the middle of the fused portion, but no sharp line of demarcation between the two halves. There is no distinct isthmus or connecting piece as figured for many cases from human pathology, although an evagination from the area of fusion projects into

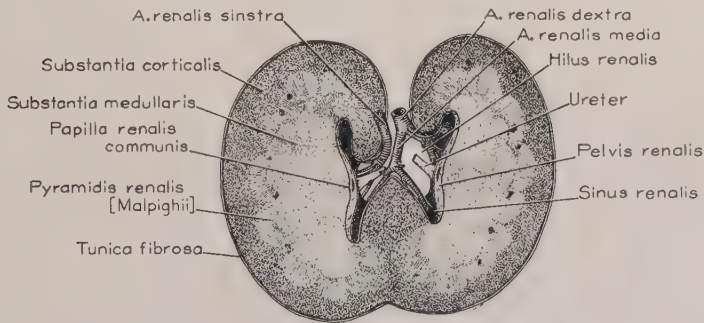


Fig. 1 Longitudinal section of horseshoe kidney, from dorsal aspect. F.M.N.H. no. 1337. $\times 1$.

the dorsal wall of the hilus. The pelves remain separate, with well-defined pyramids leading to the papillae.

The weights and measurements of the kidneys of this cat, Ekman's specimen, and a normal female are included in the following tables. Average body and kidney weights are from Latimer ('36, '39).

Available information on the development of the kidney in the cat is incomplete, but from the data on human material assembled by Pohlmann ('05), it is possible to deduce what happened in this cat.

The renal buds lie close together in the true pelvis, then diverge from the midline as they wander forward to their

definitive site. Fusion probably took place before the organs grew out of the true pelvis, as this is the only time that the two anlagen are close enough together (cf. theory of Lewis and Papez, p. 202; Boyden, '31). While the kidneys migrate, they are turning on their vertical axes, rotating approximately 90°. In the adult condition in the cat as in man the pelvis face medially. The ventrally directed pelvis in this cat indicate that fusion prevented complete rotation.

TABLE 1
Weights and ratios for normal and fused kidneys.

	BODY WEIGHT	RIGHT KIDNEY	LEFT KIDNEY	COMBINED KIDNEYS	RATIO
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	
Present specimen, adult ♀	1400+ ¹			16.1	1: 87
Ekman's specimen, adult ♂ (Angora)	3318			33	1: 100
FM no. 1288, normal adult ♀	2289	9.45	10.3	19.8	1: 115
Latimer's average of 52 ♀♀	2445.17			16.89	1: 144

¹ Minus skin, and slightly desiccated.

TABLE 2
Measurements of normal and fused kidneys.

		RIGHT KIDNEY	LEFT KIDNEY
		<i>mm.</i>	<i>mm.</i>
Present specimen, adult ♀	Length	37	35.5
	Over-all width	19.5	18
Width of long. section from lateral border to papilla		16	15.5
FM no. 1288, normal adult ♀	Length	35	34.5
	Over-all width	23	27
Width of long. section from lateral border to papilla		17.5	19

Migration of the permanent kidney is completed before the 16-mm. stage in the cat (Huntington and McClure, '20) when the permanent renal veins are developed. It is surprising that fusion did not affect the normal migration more in this case. But as in man, the fused kidney did not ascend above the inferior mesenteric artery. Ekman's case of horseshoe kidney, also at the normal level, was strikingly displaced, the entire mass lying on the right side of the body. The

present case was symmetrically displaced toward the mid-line, with slightly more of the fused organ lying on the right side of the vertebral column.

Pohlmann states that the presence of a definite line of demarcation between the two constituents shows that fusion occurred at a late stage; the more indefinite the line, the earlier the fusion. But Boyden's case ('32) of a young human embryo, and Gruenwald's recent analysis of migration ('43) indicate that fusion occurs before the 10-mm. stage. In any event since the line of demarcation in our specimen has been obliterated, fusion must have occurred at an early period.

Fusion in this cat was less extensive than in Ekman's, where the definite shape of two kidneys is more nearly lost in the form of a "cake-kidney" (Kuchenniere). The larger size of Ekman's kidney is accounted for by the difference in breeds and sex. The present cat was a rather small individual, but the weight of the fused kidney bears about the same relation to body weight as do the combined kidneys in our normal adult female. The combined kidney weight in our atypical female is almost the same as Latimer's average for fifty-two female cats, but our percentage of body weight is higher. The marked deviation from the average proportion of combined kidneys to body weight in our specimen may well be partially the result of the shrunken condition of our cat. Fusion of the kidneys apparently did not effect an increase in the amount of renal tissue usual for normal physiological functions. Linear measurements of the right and left components of the horseshoe kidney conform rather closely to those of the separate kidneys in our normal adult female cat.

THE ARTERIES

Certain anomalies in the arteries of the lumbar region are obviously correlated with the horseshoe kidney. Only those vessels that differ from the normal condition in position or distribution are mentioned.

The anomalous arterial supply of the horseshoe kidney (fig. 2) is readily explained by its developmental history.

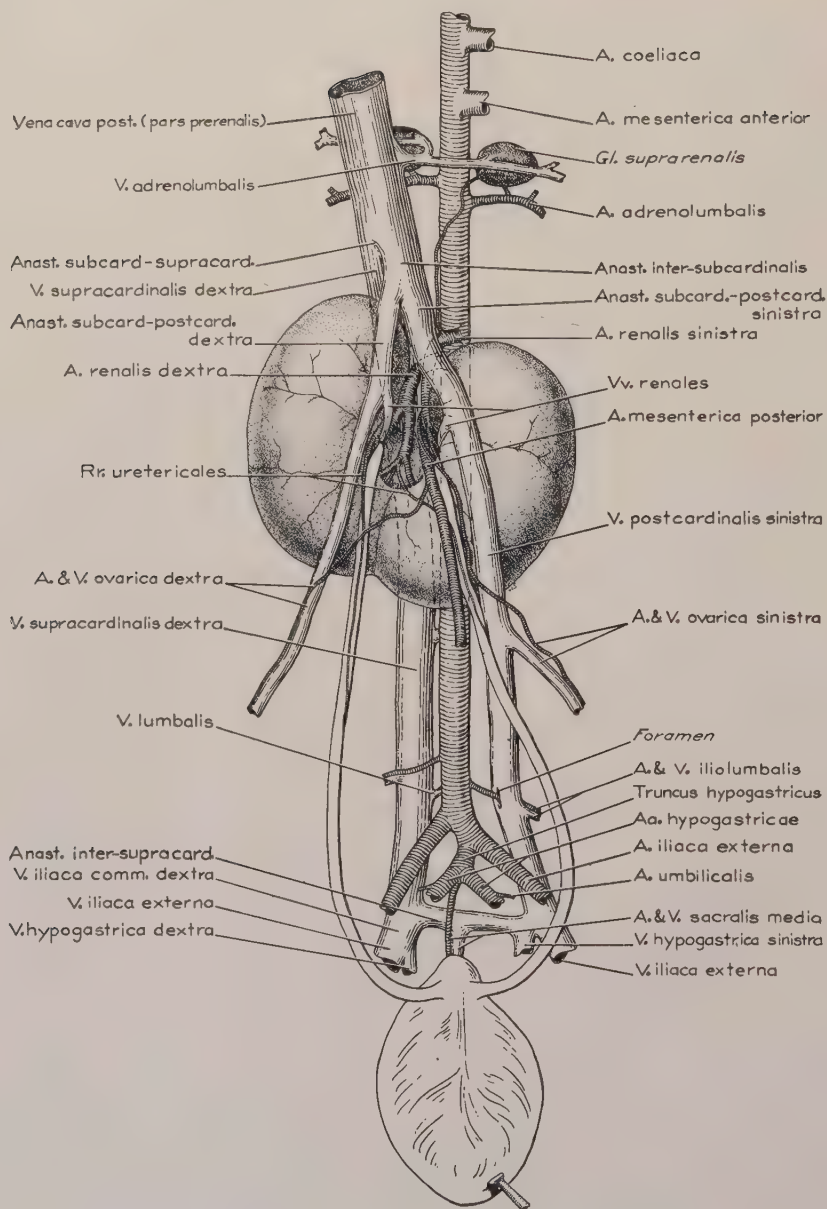


Fig. 2 Horseshoe kidney and associated blood vessels, from ventral aspect. Adipose tissue has been removed. F.M.N.H. no. 1337. $\times 1$.

Vascularization of the kidney takes place after it has completed its forward migration (Pohlmann). Therefore it may be assumed that the variations in the arteries occurred after fusion of the kidneys, and that this obstruction along the normal path of development contributed largely to the adult condition.

1. A. renalis sinistra arises from the left wall of the aorta, immediately turns upon its stem, and crosses the aorta to the

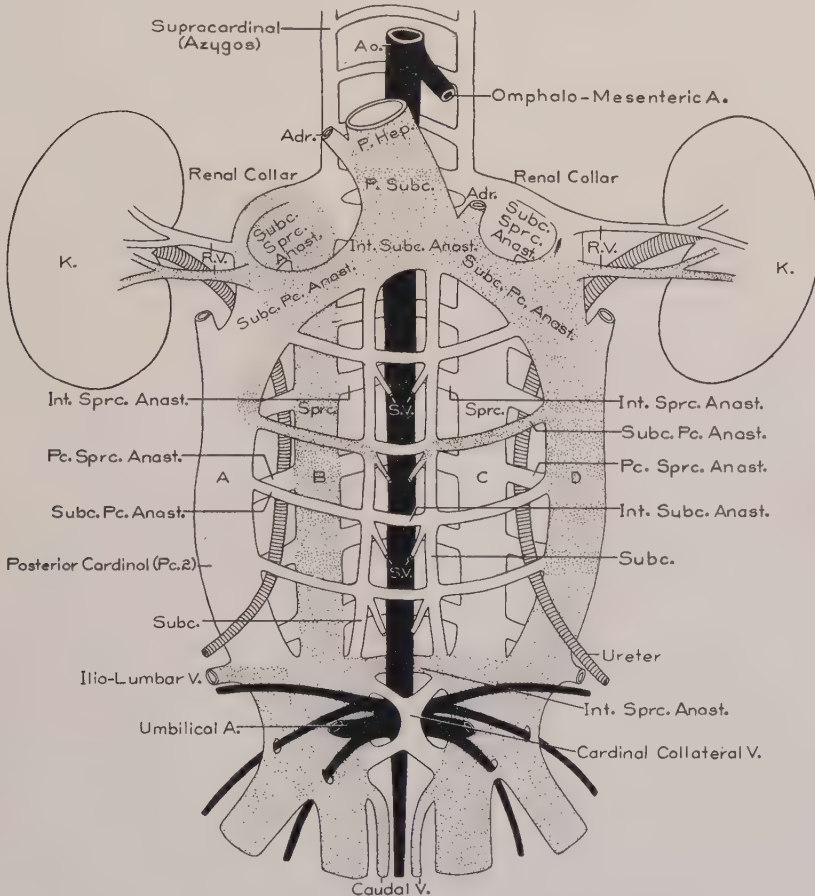


Fig.3 Composite diagram of embryonic veins of the domestic cat (*Felis domestica*), based on a detailed study of the development of the veins, after McClure and Huntington. The stippled areas, added by the writer, indicate the vessels represented in the cat with the horseshoe kidney.

right side where it courses ventrad of the right renal artery to supply the displaced left kidney. The ventrodextral situation of the left renal artery is unquestionably correlated with the dystopy of the left kidney.

2. *A. renalis dextra*, much larger than the left renal, arises ventrolaterally from the right wall of the aorta, just posterior to its fellow, and divides at the antero-ventral border of the hilus into a strong lateral branch to the right kidney, and a subequal medial branch, *A. renalis media*, that richly supplies the fused area. Fine *Rr. uretericales* are given off to the anterior ends of the ureters. The ventroposterior direction of the right renal artery is obviously due to the position of the kidney very close to the midline. Increase in caliber is in ratio to the increased area to be supplied. Subdivision of the right renal artery takes care of the fused part of the kidney. Supernumerary arteries to the connecting piece have been reported. The right renal artery in Ekman's cat also was subdivided into an artery for the right kidney proper and one especially for the isthmus.

3. *A. mesenterica posterior* arises below the right renal artery, dorsal to the left kidney, at the level of the fourth lumbar vertebra, 61.5 mm. craniad of the bifurcation of the aorta. In a normal adult female, FM 1288, this artery arose far posteriorly, at the level of the sixth lumbar vertebra, 23 mm. anterior to the bifurcation. The posterior mesenteric artery gives origin to the left ovarian artery dorsal to the horseshoe kidney, winds through the left half of the hilus, gives off the right ovarian artery as it leaves the hilus and courses ventrally over the posterior extremity of the left kidney to its normal distribution.

The far anterior position of the posterior mesenteric artery is correlated with fusion of the kidneys in the path of its descent. In man the inferior (posterior) mesenteric migrates from the twelfth thoracic to the third lumbar segment. From what is known of the arterial development of the cat, a similar migration occurs, and the fused kidney in this case prevented complete migration. Ekman reported that the

posterior mesenteric artery was held in its anterior position by pressure of the renal vessels, and that the right internal spermatic artery had a common origin with it. Both arteries of the gonads arise from the posterior mesenteric in this specimen, a variation along much the same line. During the ascendancy of the mesonephros as the urinary organ, many mesonephric arteries arise ventrolaterally from the aorta, and are extremely variable. The ovarian arteries are normally derived from the mesonephric vessel opposite the second lumbar vertebra. It has been shown by Broman ('08) that union of a mesonephric artery with a nearby ventral aortic branch is not uncommon, and this may explain the origin of the ovarian arteries from the incompletely descended posterior mesenteric artery in the present case.

THE VEINS

Variation in the veins is more extensive than in the arteries, and may be traced to a wholly different cause not directly related to fusion of the kidneys.

McClure and Huntington ('29) demonstrated that all atypical conditions of the postcava in the adult cat can be explained as modifications of a common ontogenetic plan, and that there are seventeen predictable forms of atypical arrangements. The present specimen (figs. 2, 3) conforms basically to their type BD, which appears to be a rare anomaly. Darrach ('07) found two similar cases among 605 cats (0.3%), and McClure and Huntington added a third (embryonic) instance. Type BD is characterized by persistence of the right supracardinal and left postcardinal veins. The present specimen exhibits additional less conspicuous anomalies in the postcaval circulation; as is shown in figure 3, these may all be easily interpreted as persistent vestiges of parts of the embryonic pattern.

The important details of the postcaval pattern in this individual are:

1. The unpaired prerenal division (pars subcardinalis) of the vena cava posterior is formed by the union of two caval

veins of unequal caliber. This union takes place 6 mm. anterior to the kidney, at the level of the third lumbar vertebra. This prerenal division is the only typical constituent of the postcaval system in this specimen.

2. V. supracardinalis dextra, the larger of the paired caval veins, arises from the right iliac confluence and passes forward dorsad of the kidney. At its origin it is connected with the left postcardinal vein by a strong anastomotic branch, then receives the right iliolumbar vein and four lumbar veins, and finally empties into the prerenal postcava. Its position dorsad of the kidney and ureter throughout its course, and its distribution chiefly to the right side, indicate that this vein is the persistent right supracardinal.

3. V. postcardinalis sinistra is the direct continuation of the left external iliac vein. It courses diagonally ventromesad, at first dorsal to the left external iliac artery, then lateral to the aorta, finally shifting ventrad of the aorta and kidney. Immediately anterior to the kidney it unites with the right subcardinal-postcardinal anastomosis and continues via the intersubcardinal anastomosis into the prerenal postcava. The left postcardinal receives the left iliolumbar, ovarian (representing the left subcardinal), and renal veins, in addition to the strong anastomotic ramus connecting it with the right supracardinal. The relationship of this vein to the kidney, ureter, and aorta identifies it as the persistent left postcardinal.

4. The right ovarian and renal veins unite ventrad of the hilus of the kidney; the combined trunk represents the embryonic subcardinal-postcardinal anastomosis. The right subcardinal-postcardinal anastomosis joins the left subcardinal-postcardinal anastomosis immediately anterior to the kidney, forming the short intersubcardinal anastomosis that represents the ventral part of the embryonic renal collar. The intersubcardinal anastomosis unites with the right supracardinal vein, in the subcardinal-supracardinal anastomosis, that flows into the single prerenal division (pars subcardinalis) of the vena cava posterior.

5. V. iliaca communis sinistra is absent. This condition was discussed by McClure ('00), who considered it to be a result of the persistence of the left postcardinal vein.

6. A strong transverse anastomosis connects the right supracardinal and left postcardinal, posterior to and dorsad of the bifurcation of the aorta in the minor pelvis. This anastomotic vessel receives the left hypogastric and middle sacral veins. It represents one of the lower intersupracardinal anastomoses of the embryonic venous system.

7. The left postcardinal vein is perforated by a foramen anterior to the iliolumbar vein; this perforation transmits the left iliolumbar artery. McClure mentioned foramina in veins for the transmission of either arteries or nerves as a common anomaly in the domestic cat, and Darrach found vessel islands at the level of the iliolumbar vein in 2.7% of 375 domestic cats.

SUMMARY

The kidneys

1. The kidneys are fused at their posterior extremities to form a horseshoe.

2. Evidence points to an early date of fusion.

3. Normal migration of the kidney occurred, but rotation was incomplete.

4. Both kidneys lie closer to the vertebral column than normal, and the fused organ is slightly displaced dextrad.

5. Ureters, suprarenals, and genitalia are normal.

The arteries

1. The renal arteries are displaced proportionately to the dystopia of the kidneys.

2. A large branch of the right renal artery supplies the fused area, as has been frequently reported in cases of kidney fusion.

3. Both ovarian arteries arise from the posterior mesenteric artery, one of the potential variations of the embryonic vascular system.

The veins

1. The embryonic left postcardinal vein has been retained in addition to the normal retention of the right supracardinal as the postrenal vena cava posterior.

2. The right subcardinal-postcardinal anastomosis has persisted.

3. An anastomotic vessel, lying posterior to and dorsad of the bifurcation of the aorta, represents one of the lower intersupracardinal anastomoses of the embryo.

4. The left common iliac vein is absent.

5. A foramen for the left ilio-lumbar artery pierces the left postcardinal vein.

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STIMULATION OF NEPHROGENIC TISSUE BY NORMAL AND ABNORMAL INDUCTORS

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THREE TEXT FIGURES AND TWO PLATES (TEN FIGURES)

Experimental obstruction of the growing wolffian ducts of chick embryos has revealed that the mesonephric blastema undergoes its normal differentiation only when stimulated by that duct (Boyden, '27; Gruenwald, '37; Waddington, '38). A similar relation was found to exist between the metanephric blastema and the ureteric bud. In a recent series of experiments ('42 a) evidence was shown of the spatial limitation of the nephrogenic potency in the mesenchyme, corresponding roughly with what is known in descriptive embryology as the nephrogenic tissue. The same experiments raised two additional questions which could not be definitely answered at that time. Findings in several embryos suggested that nephrogenic tissue may be stimulated to differentiate into nephrons not only by the wolffian duct or the ureteric bud, but also by nervous tissue. Other observations reported at that time indicated that the metanephric blastema may give rise to mesonephric nephrons. The present report deals with new experiments bearing on these two problems. Corresponding findings in spontaneous malformations will also be described. For a discussion of the related literature the reader is referred to the author's earlier reports ('37, '42 a). Finally the occurrence of external glomeruli on the mesonephros will be briefly reported. Such glomeruli had previously ('42 a) been found in experimental embryos, but it could not be decided whether or not their presence was normal or related to the experiment.

EXPERIMENTAL METHOD

The technique used in the present series is very similar to that previously employed ('42 a); and will therefore not be described in detail. Chick embryos of the latter part of the second day were operated upon, hosts and donors being of the same age. As in the earlier experiments, grafts were prepared containing the neural tube and whatever of the adjacent organs could not be separated from it. Greater care was taken in the new series to denude the nervous tissue as completely as possible of the surrounding mesenchyme, in order to bring it in direct contact with the tissues of the host. These grafts were placed in a transverse cut at the level of the "segmental zone" of the host, within the lateral half of the prospective somite material and the adjacent parts of the intermediate and lateral mesoderm. However, in the present experiments the grafts were placed somewhat farther caudally than in the previous series. At the time of the operation, the distance of the graft from the last somite in the host was equal in length to approximately six to eight differentiated segments; the number of somites subsequently developing in that area may have been greater than that.

This site was chosen in order to bring the grafted nervous tissue in contact with the metanephric blastema, and at the same time eliminate the ureteric bud by obstructing the path of the growing wolffian duct. The metanephric blastema was tested because previous experiments had shown that this tissue, in contrast to the mesonephric blastema, never differentiates into epithelial structures in the absence of an organizer (Boyden, '32; Gruenwald, '42 a). An inconvenience connected with this site of the graft was the formation of an excessive hydronephrosis of the mesonephros on the side of the operation because of the far caudal obstruction of the wolffian duct. The dilatation of the duct resulted in slight disturbances in the location of adjacent organs.

Also another attempt was made to demonstrate the influence of nervous tissue upon the nephrogenic blastema. The tissue of the prospective somites at the level of the metanephric

blastema was destroyed by electrolysis or excision in the hope that nervous and nephrogenic tissues would then develop in close contact. This experiment had to be done in 2-day embryos because the bleeding from large intraembryonic vessels would be too severe in later stages. It turned out that the destroyed mesoderm is regenerated with remarkable speed and perfection and 2 days after the operation all derivatives of the somites appear completely normal, even in cases in which the damage had been sufficiently severe to cause permanent disturbances of the adjacent neural tube. (The experiments were done in vitally stained embryos so that there can be no doubt in correct localization of the original defect.) Thus the desired contact of the tissues to both sides of the somites was never accomplished in these experiments.

All embryos were inspected twice daily through the cellophane window covering the opening in the egg shell; dead embryos could thus be detected in time to be used for histological examination. However, most of the embryos survived up to the time designated for the termination of the experiment. All embryos were fixed in Bouin's fluid and cut serially in transverse direction. Azan stain was used in all instances. Graphic reconstructions of some of the experimental embryos were prepared from the sections, and finished in the same manner as those shown in the earlier report ('42 a). The midline was arbitrarily taken as a straight line, and all distances measured with an eyepiece micrometer. The structures shown in these reconstructions appear projected on a frontal plane, at an original magnification of 100 (reduced in figs. 1-3).

OBSERVATIONS

Experiments

Of thirteen host embryos reaching the desired age of approximately 5 days, six showed evidence of stimulation of the nephrogenic blastema by the grafted nervous tissue. Three of these will now be described.

Embryo 817 was operated upon after 1 day and 23 hours of incubation, in the 23-somite stage. A graft taken from a

20-somite embryo of the same age was introduced in the above described position. The living host embryo was exposed after 5 days and 4 hours of incubation and fixed. Figure 1 shows a reconstruction of the caudal part of the urinary organs and the graft. Normal conditions prevail on the left side.

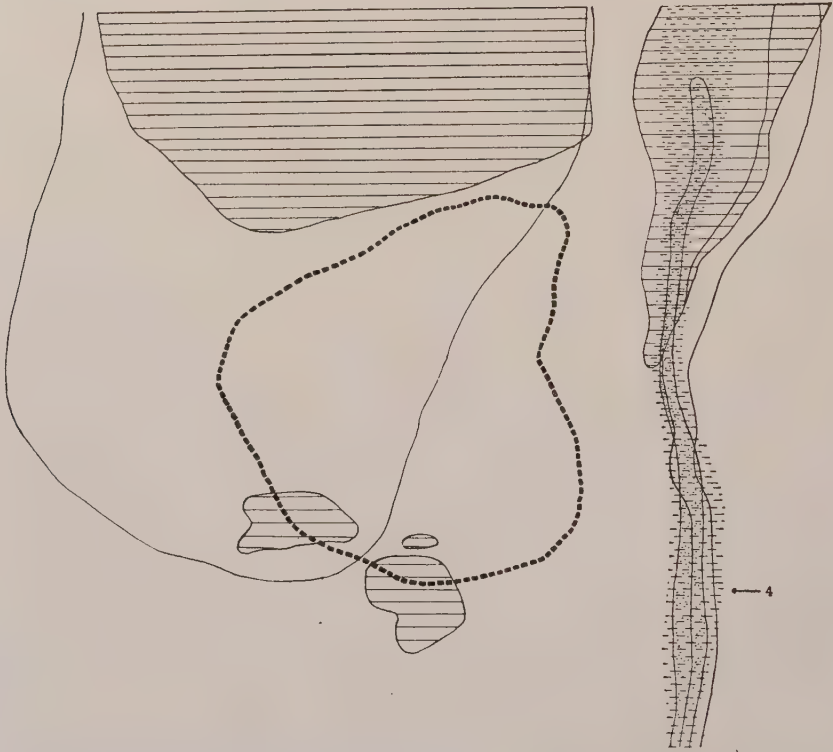


Fig. 1 Graphic reconstruction of the urinary organs of embryo 817. Wolffian ducts: thin outlines; mesonephroi: horizontally lined; ureteric bud: stippled; adjacent metanephric blastema: broken horizontal lines; grafted tissues: broken heavy lines. These structures appear projected on a frontal plane. The arrow indicates the level of the corresponding photograph of a section. Drawn at $\times 100$; present magnification 47.

On the right side the greatly dilated blind end of the wolffian duct may be seen. The mesonephros, spread out over the circumference of this sac, occupies an unusually large area on frontal projection without being enlarged in its mass.

Three additional areas of mesonephric tissue are indicated at the level of the caudal end of the wolffian duct and the graft. Of these, the lateral one is located dorsal to the wolffian duct, and in closest proximity to nervous tissue of the graft. It is highly probable that the differentiation of this tissue was stimulated by grafted nervous tissue, but the proximity of the wolffian duct does not allow a definite conclusion in this instance. The large caudal area of mesonephric tissue indicated in figure 1, is in equally close relation to the nervous tissue, but so far distant from the wolffian duct that induction by the latter can be ruled out. Figure 4 shows this area as containing well differentiated epithelial tubules; no glomeruli were found. Because of a dislocation due to the hydro-nephrosis and the graft, it can not be determined with certainty which position this tissue would have occupied under normal conditions. However, its location caudal to the level of normal mesonephros formation, and distant from the coelomic cavity makes it probable that metanephric tissue had undergone the differentiation. The same section shows the metanephric blastema on the left side in normal relation to the ureteric bud. This blastema shows no trace of epithelial differentiation and would normally remain in a very similar condition during several more days of incubation.

A similar experiment was performed on embryo 818 in the 25-somite stage, after 1 day and 23 hours of incubation. The living embryo was fixed at an age of 5 days and 4 hours. A frontal reconstruction (fig. 2) again shows normal conditions on the left side, and on the right side the graft and cranial to it the obstructed and dilated wolffian duct, with a mesonephros ending at approximately the same level. The reconstruction also indicates the presence of two groups of tubules far from the wolffian duct. These are shown on section in figures 5 and 6. Both are situated in close proximity to the transplanted nervous tissue. In this case the tubules are found in the typical location of the metanephric blastema,

as shown by a comparison with the normal left side. The structure of the tubules and the normal left primordium is very similar to that described in embryo 817.

Another example of the same type of experiment is embryo 820. It had been operated upon in the 27-somite stage, after 2 days of incubation, and fixed at an age of 5 days and 4 hours.

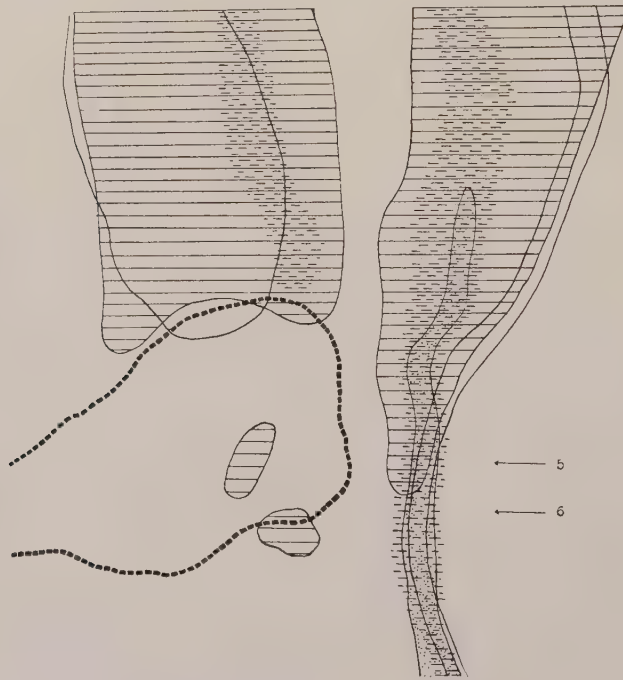


Fig. 2 Graphic reconstruction of the urinary organs of embryo 818, shaded as figure 1. Drawn at $\times 100$; present magnification 47.

A reconstruction (fig. 3) again shows considerable hydro-nephrosis of the right mesonephros cranial to the transplant. Near the caudal border of the grafted tissues (fig. 7), an isolated group of tubules of the mesonephric type is present in the characteristic position of the metanephric blastema (fig. 8).

Malformations

Three chick embryos with malformations were mentioned in a previous report ('42 a) as suggesting induction of nephrons by nervous tissue. In these embryos malformations of various types were present incidental to the experiments performed, and had resulted in unusually close proximity

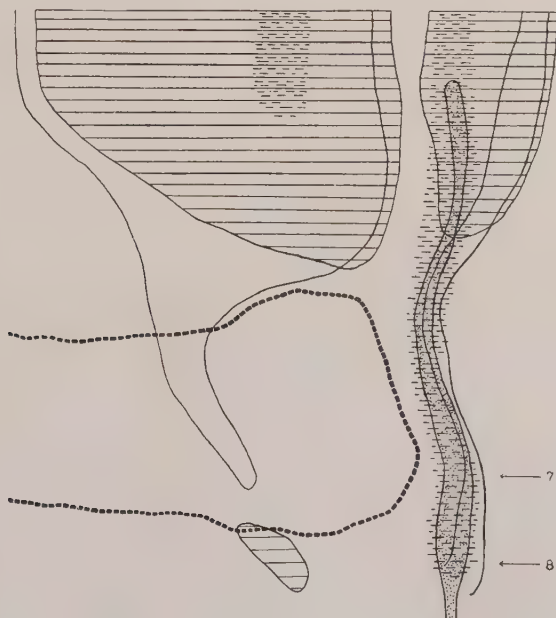


Fig. 3 Graphic reconstruction of the urinary organs of embryo 820, shaded as figure 1. Drawn at $\times 100$; present magnification 47.

of nervous and nephrogenic tissues. The only possible relation of these observations to the experiments performed on the same embryos, is that in two of them unusually large amounts of unstimulated nephrogenic tissue were present and able to respond, as a consequence of obstruction of the wolffian duct. The third case of that group will now be described in more detail, along with significant findings in other malformed embryos which developed undisturbed by experimental procedures.

On the maldeveloped experimental embryo 773 (see also Gruenwald, '42 a, figs. 6 and 24) an operation similar to the one of the present series had been performed on the right side. On the left side, not directly affected by the experiment, a distortion of the axial organs and apparent absence of the somite derivatives had brought the neural tube and spinal ganglia into close proximity to the nephrogenic tissue, and groups of tubules are present dorsal to the normal mesonephros, probably arising from the metanephric blastema. One of these groups is shown in figure 9. One tubule shown in this figure, winds around a spinal ganglion and ends in contact with the nervous tissue of the spinal cord.

In an embryo of the latter half of the first week with numerous severe malformations (F 201), absence of the notochord and associated abnormalities of the nervous system are responsible for abnormally intimate relations of nervous tissue with the metanephric blastema. A mesonephric tubule was found leading from the dorsal boundary of the mesonephros through the area of the future metanephros, and ending in close contact with an abnormal accumulation of nervous tissue (fig. 10). An organizing influence of the nervous tissue is suggested in figures 8 and 9 not only by the close proximity of the tissues in question, but also by the arrangement of the tubules which lead toward the nervous tissue in a similar manner as they would normally do toward the wolffian duct. Several more epithelial tubules and cysts resembling mesonephric structures, were found in the metanephric blastemas of the same embryo near dislocated masses of nervous tissue.

A third embryo (F 243) fixed after 6 days and 2 hours of incubation, show extensive mesonephros differentiation in its left metanephric blastema, in connection with a distortion of its axial organs. The malformation of the region under consideration is, in its essential traits, similar to that of embryo 773. The spinal cord is twisted so that its left side with the corresponding ganglia faces ventro-laterally. The proximity to the metanephric blastema is brought about by

this torsion as well as a reduction or absence of the somite derivatives in this region. Several well developed mesonephric tubules are found dorsal to the mesonephros proper. Some of these are shown in figure 11. They were followed on serial sections and showed no communications with tubules of the mesonephros or the wolffian duct. Some of them are connected with dilated renal corpuscles with poorly developed glomeruli. In one instance an oblong corpuscle with several capillary tufts was seen. This may be an indication of retention of secreted fluid. The characteristic attachment of the ends of these tubules to the spinal cord as described and pictured in the preceding observations was found several times in this embryo as well.

Including the descriptions given in the previous publication ('42 a), five chick embryos have been found to date, in which spontaneous malposition of the nervous system led to induction of mesonephric differentiation in the nephrogenic tissue. In the three cases described in the present report, the metanephric blastema is involved. The comparatively large incidence of these findings in the author's material may appear surprising. However, it must be remembered that these differentiations are inconspicuous, and careful investigation may be necessary to distinguish them from the nearby tubules of the normal mesonephros. Examination of maldeveloped embryos with these facts in mind will doubtless reveal additional cases.

External glomeruli on the mesonephros

Six chick embryos of the previous experimental series ('42 a) showed either one or two external glomeruli attached to the ventral surface of the mesonephros. These glomeruli were found on the well developed and apparently functional portion of the organ, definitely too far caudally to be related to the pronephros. No conclusions were drawn from these findings since it was not known whether or not external glomeruli occur on the mesonephros of normal embryos.

At the present time serial sections of more normal chick embryos are available to the author for comparison. Most of these show the well-known external glomeruli of pronephric origin attached to the cranial, poorly developed and regressing portion of the mesonephros. In addition, external glomeruli were found in three out of twenty-one normal embryos of the fifth to seventh day, as well as in one maldeveloped embryo with anomalies not affecting the mesonephros and its drainage. In one of the normal embryos (4 days 17 hours) two external glomeruli are present on the right mesonephros, one at the level of the ostium of the müllerian duct and the other one 740 μ further caudally, at the level of liver and stomach (fig. 12). The above mentioned maldeveloped embryo of 5 days shows an external glomerulus with a nephrostome on its left mesonephros, caudal to the liver and cranial to the gonad primordium. Figure 13 shows this glomerulus and the nephrostome leading into a duct which joins a normal tubule near its internal glomerulus.

To the best of the author's knowledge, external glomeruli have not been described in the literature as occurring in regions of the mesonephros where no pronephros ever developed. The author's own findings concern embryos of the fifth and sixth day; only one apparently degenerating external glomerulus was found in a normal embryo of 6 days and 7 hours. Nephrostomes occur in association with these glomeruli, but not in all cases. The material at hand is not sufficiently large to allow a full description of origin, fate and frequency of these structures.

It might be of interest in this connection to recall that glomerulus-like formations have been described as occurring regularly on the dorso-lateral surface of the mesonephros in human embryos of about 14 to 21 mm. (Gruenwald, '42 b). However, it is highly problematic whether these structures are real glomeruli, and comparable with those just described in chick embryos.

DISCUSSION

It is evident from the present findings that the metanephric blastema which had been shown not to differentiate independently into nephrons (Gruenwald, '37, '42 a), forms epithelial tubules when in contact with nervous tissue. These tubules are, in their structure as well as in their rate of differentiation, similar to mesonephric rather than metanephric tubules; not all of them are connected with renal corpuscles. The present findings confirm two conjectures derived from incidental findings in previous experiments ('42 a), namely stimulation of specific differentiation of nephrogenic tissue by nervous tissue, and the capacity of the metanephric blastema to undergo mesonephric differentiation. The former had been suggested by the conditions in mal-developed experimental embryos in which the experiment had left part of the nephrogenic tissue devoid of its physiological inductor, and the malformation had brought that tissue in contact with the nervous system. Also, a case was described in which an unusually large group of mesonephric nephrons was present in close proximity to transplanted nervous tissue, and in the absence of the wolffian duct ('42 a, embryo 773). These previous and present observations are evidence of the capacity of nervous tissue to induce the differentiation of nephric tubules in the nephrogenic tissue. The present observations in experiments and malformations do not indicate whether all of the embryonic nervous tissue is capable of inducing kidney tubules, or only certain parts of it. In most cases the nervous tissue near the induced tubules is irregularly developed, so that no definite conclusions can be drawn from the present evidence.

The process just discussed is by no means the first known example of inducing powers apparent in experiments and never active in the course of normal development. Limb bud formation, for instance, may be induced by otic vesicles, nasal pits, and even foreign bodies inserted in proper places. For a discussion of this problem see Weiss ('39).

The second fact brought out by the present findings is the apparent capacity of the metanephric blastema to undergo differentiation of the mesonephric type. This had previously been suggested by findings in one experimental embryo ('42 a, embryo 724) in which the wolffian duct, after passing through a transplant, had grown dorsad and reached the metanephric blastema. There it was joined by a group of tubules corresponding in their state of differentiation to the mesonephroi of the same embryo. The nervous tissue serving as inductor in experiments and malformations is, in its action upon the metanephric blastema, similar to the wolffian duct rather than the ureteric bud. It seems that the inductor determines whether the metanephric blastema is to follow the mesonephric or the metanephric type of development. The facts at hand do not allow a further analysis of this "selective" induction. A possible explanation is, that a difference in either intensity or time of induction determines the type of differentiation to be followed. Stronger or earlier induction might induce the rapid mesonephric type whereas weaker or later action of the inductor would lead to slow metanephric differentiation. This hypothesis eliminates all difficulties in classifying the process in question according to the directions set forth by Weiss ('39). This author draws a definite line between activation brought about by an inductor, and a pattern not necessarily determined by the inductor, which is responsible for the quality of the response. Weiss quotes examples in which only the activation and not the pattern is furnished by an implanted inductor, and others in which both these activities can definitely be traced to the inductor introduced by an experiment. It is probable that the induction under consideration belongs to the former type since the elicitation of just the nephric type of differentiation can hardly be credited to a pattern determined by the nervous tissue. The seemingly selective action in inducing mesonephric rather than metanephric differentiation in the metanephric blastema would then be a mere consequence of the intensity or time of induction. Attempts will be made to test this conjecture in future experiments.

It had been found earlier ('42 a) that nephron formation in response to induction by the wolffian duct or the ureteric bud is limited approximately to what is known from descriptive embryology as nephrogenic tissue. The fact that nervous tissue acts as an inductor in a similar manner as the wolffian duct, is further evidence of the spatial limitation of the nephrogenic potency. Large amounts of mesenchyme never differentiate into nephrons although they are in contact with nervous tissue.

During the second week of incubation the metanephros of the chick embryo expands considerably and is then found in closest proximity of ganglia and nerve trunks. Although part of the metanephric blastema is yet undifferentiated or in the process of differentiation, no influence of the nervous tissue can be noticed. It cannot be decided which changes of the tissues involved account for this lack of stimulation or response. In experimental embryos with absence of one of the ureteric buds (Gruenwald, '37, '42 a) the unstimulated metanephric blastema does not expand sufficiently to be in contact with nervous elements. These embryos can therefore supply no additional information.

CONCLUSIONS

The present findings, in conjunction with the results of previously reported experiments ('37, '42 a), allow the following conclusions regarding distribution and activation of the nephrogenic potency in the chick embryo. Differentiation of mesonephric and metanephric nephrons is normally induced by the wolffian duct and the ureteric bud, respectively. In the absence of this physiological inductor the metanephric blastema never shows specific differentiation, whereas in some cases the mesonephric portion independently differentiates into more or less vestigial nephrons. Mesenchyme other than that of the nephrogenic blastema does not respond by nephron formation when in contact with the above mentioned inducers. Nervous tissue has an inducing effect upon nephrogenic tissue similar to that of the wolffian duct. The metanephric blastema undergoes the fast mesonephric instead of the slow

metanephric type of differentiation when in contact with either the wolffian duct or nervous tissue. This suggests that the potencies of the mesonephric and metanephric blastemas are very similar if not identical, and that the inductor determines the type of differentiation to be followed. A difference in intensity or time of induction is considered as a possible cause of this selective action of the inductor.

It is impossible at the present time to tell whether or not the pronephric blastema conforms with the other portions of the nephrogenic tissue in its potencies. Its ability of self-differentiation as well as the vestigial character of the pronephros in the chick embryo, would render experimental investigation of this question extremely difficult, if not impossible.

SUMMARY

The right metanephric blastema of chick embryos was exposed to the influence of grafted nervous tissue instead of its normal inductor, the ureteric bud. The blastema responded by forming tubules equal in structure and rate of differentiation to those of the mesonephroi. Similar observations were made in spontaneous malformations. The nervous tissue is, in its inducing action upon nephrogenic tissue, very similar to the wolffian duct. These experiments and malformations also confirm an earlier suggestion that the metanephric blastema is capable of both the mesonephric and metanephric type of differentiation, and that the mesonephric and metanephric portions of the nephrogenic tissue are potentially alike or very similar.

The occurrence of external glomeruli on the mesonephros caudal to the pronephric region is briefly described. These glomeruli may be found in otherwise normal chick embryos of the fifth and sixth day.

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PLATE 1

EXPLANATION OF FIGURES

Sections of chick embryos after implantation of nervous tissue in the metanephric region.

4 Embryo 817.

5 and 6 Embryo 818.

7 and 8 Embryo 820.

I, implant; M, metanephros of normal left side; T, induced tubules. Azan stain. The levels of all sections are indicated by arrows in the respective reconstructions (figs. 1-3).

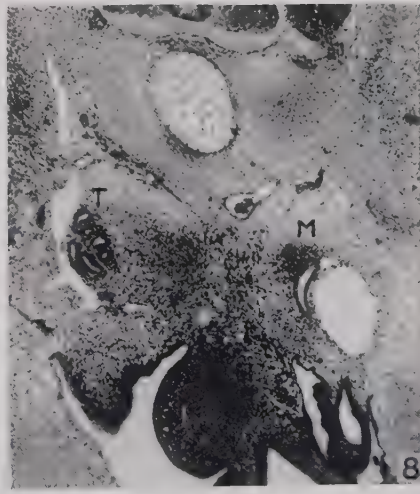
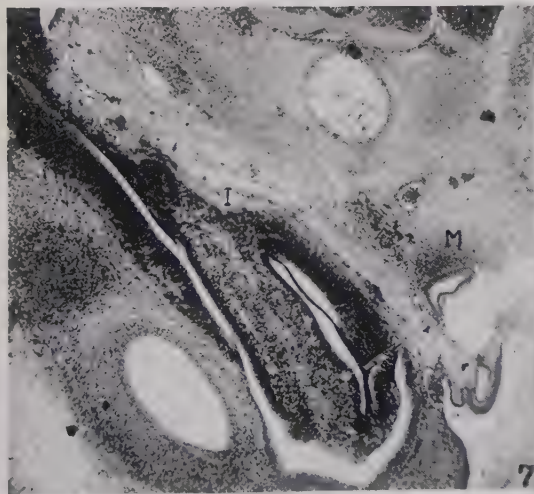
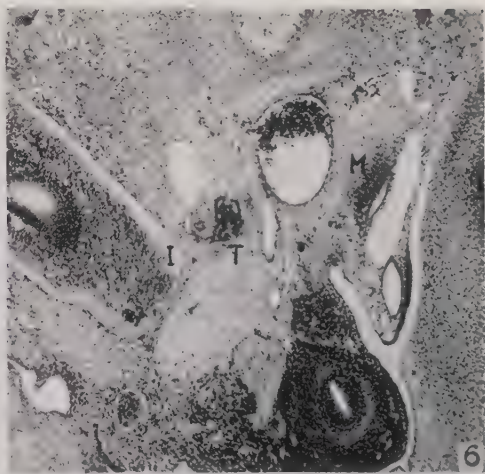
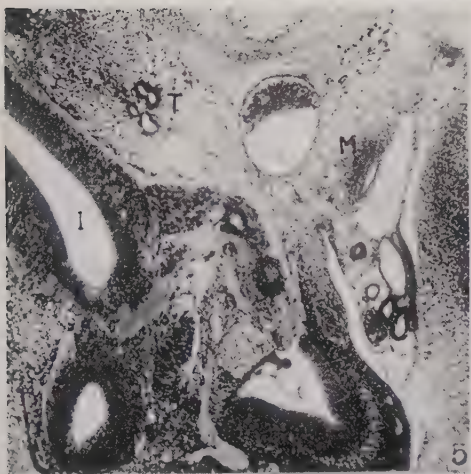
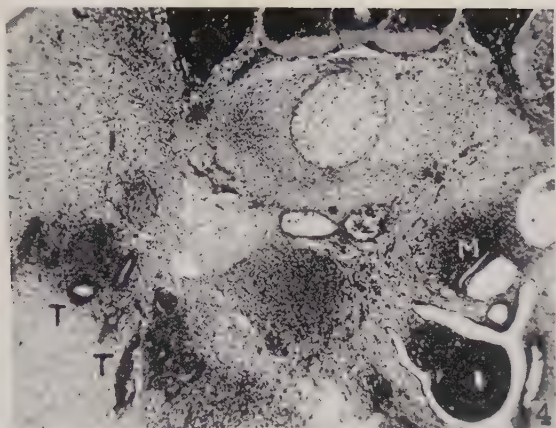


PLATE 2

EXPLANATION OF FIGURES

Sections of chick embryos in which the metanephric blastema shows the effect of contact with nervous tissue, due to spontaneous malformations.

9 Experimental embryo 773 (see also Gruenwald, '42 a) with incidental malformations. The metanephric blastema has differentiated into tubules (T). One of these partly surrounds a spinal ganglion (G) and terminates in contact with the neural tube. M, mesonephros, Azan stain.

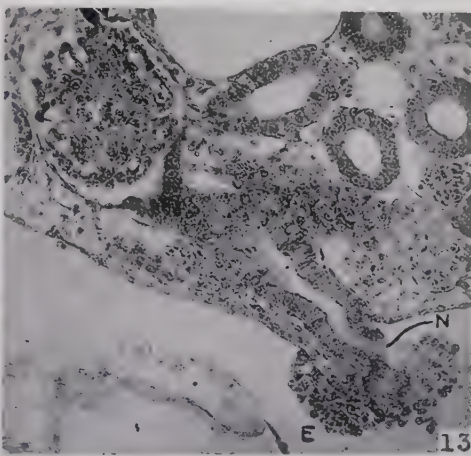
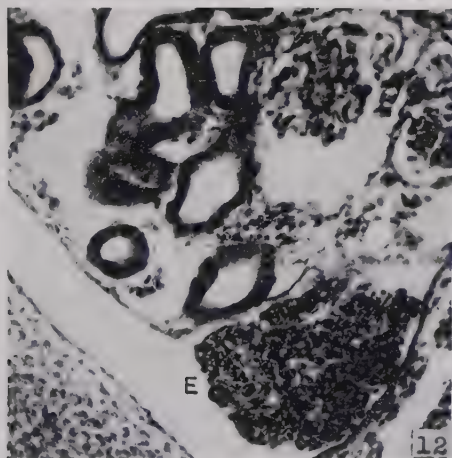
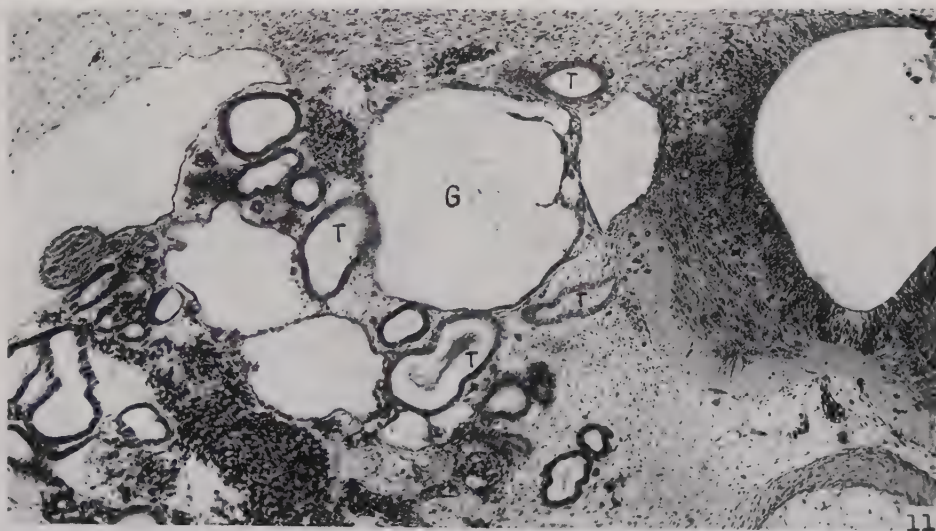
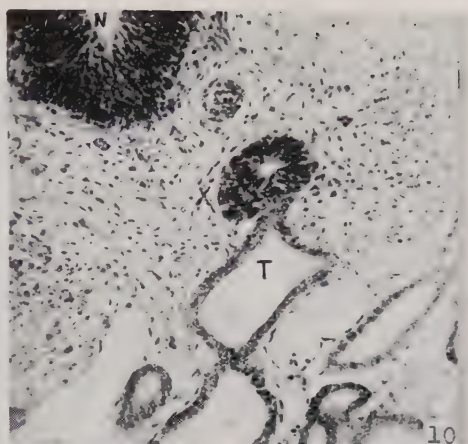
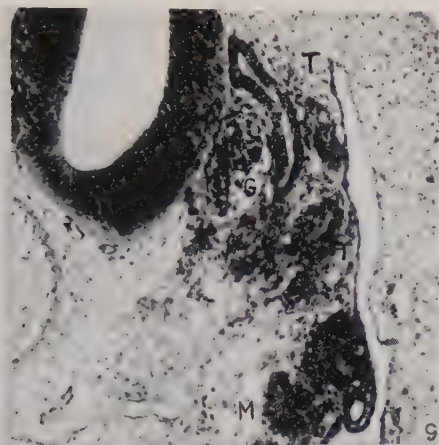
10 Embryo F 201. A mesonephric tubule (T) emerges dorsally from the mesonephros and joins an abnormal accumulation of nervous tissue (X) in the region of the metanephric blastema. N, neural tube. Azan stain.

11 Embryo F 243, age 6 days and 2 hours. The left side of the spinal cord and adjacent ganglia (G) are in contact with mesonephric tubules (T) differentiating dorsal to the normal mesonephros in the metanephric blastema. Azan stain. (The ganglion on this section shows a cystic deformity as does the adjacent part of the spinal cord.)

Sections of chick embryos showing external glomeruli on the mesonephros, caudal to the pronephric region.

12 Normal embryo, age 4 days and 17 hours. E, external glomerulus. Azan stain.

13 Embryo F 247, age 5 days. with malformations not affecting the urinary organs. An external glomerulus (E) is associated with a nephrostome (N) from which a tubule may be traced on tangential section to its junction with a normal mesonephric tubule. Azan stain.



REPRODUCTIVE CAPACITY OF ADULT FEMALE RATS TREATED PREPUBERALLY WITH ESTROGENIC HORMONE¹

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TWO PLATES (ELEVEN FIGURES)

Several investigators have emphasized one or another of various defects observed after prolonged administration of estrogenic hormones to female rats and mice. Treatment begun during the latter part of the prepuberal period, or after maturity, has produced pathologic changes in the upper genital tract (McEuen, '36; Gardner and Allen, '37; Zondek, '37; Korenchevsky and Hall, '40; and Burak, Wolfe and Wright, '42) and cessation of ovarian cycles followed by ovarian atrophy (Selye, Collip and Thomson, '35; Selye and Friedman, '40; and Mark and Biskind, '41). Although the effects of estrogenic hormone administration during early postnatal life have not been studied, it was previously shown that the androgenic hormone, testosterone propionate, injected in relatively large doses over a 28-day period, starting on either the first, fifth, or tenth postnatal days, caused severe reproductive deficiencies in later life (Wilson, Young and Hamilton, '40, and Wilson, Hamilton and Young, '41). Cyclic estrus did not occur and none of the animals became pregnant

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when caged with normal males. The ovaries were small and contained some normal-appearing follicles, numerous cystic and atretic follicles but no corpora lutea. The uteri were small, showed many pathologic changes, and responded poorly to injected estrogen. Attempts to produce mating behavior with estrus-inducing hormones were successful only when extremely large doses were used.

To determine the extent to which similar early postnatal treatment with estrogen might also modify reproductive capacity of the adult animal, the following experiments were undertaken.

METHOD

Prepuberal treatment with estrogen, estradiol dipropionate,³ consisted of a standard injection procedure over a period of 28 days. The desired total dose was divided into 12 equal parts, each part dissolved in 0.05 cc. of peanut oil and injected subcutaneously three times a week for 4 consecutive weeks. This procedure varied only in the total quantity of hormone administered and the age of the animal when the treatment was begun.

Thirty-two female albino rats were injected with estradiol dipropionate in total doses ranging from 0.037 to 2.4 mg., beginning within 24 hours after birth. Thirty-three additional females were each injected with a total dose of 1.2 mg., but the initial injection was made at various ages between birth and puberty in six different groups of animals. The ages selected were the fifth, tenth, fifteenth, twentieth, thirtieth and fortieth days. Twelve other females were ovariectomized within 24 hours after birth and immediately thereafter given the first injection of a routine series totaling 1.2 mg.

Injected control animals were prepared by treating eleven females according to the standard procedure with pure peanut oil, beginning the first day. A group of normal animals was ovariectomized at 3 months of age to be used as untreated controls.

³ Estradiol dipropionate was kindly supplied by Ciba Pharmaceutical Products, Inc., Summit, N. J.

The treated animals were not studied until they were 90 to 130 days of age and those in which treatment was started late in the prepuberal period were not studied until at least 2 months after the last injection. Puberty occurs about the fiftieth day in a majority of females of the strain used in these experiments. Records of body growth were not kept. When the animals were killed at the age of 4 to 5 months, the average weight was 209 gm. (range, 165–250), which was only slightly less than the 220 gm. average of normal females of comparable age. It is well known that estrogen administered to young animals causes a depression of the growth rate; but Spencer, D'Amour and Gustavson ('32 a) and Deansley ('39) have demonstrated that cessation of the treatment is soon followed by recovery from the stunted condition.

Reproductive capacity in the adult animals was tested as follows. The occurrence of cyclic manifestations of estrus was first determined by making continuous observations for estrous behavior for a period of 12 days. This consisted of attempting to elicit the copulatory reflex (Blandau, Boling and Young, '41) by manual stimulation on the back and pudendal region every 2 hours, day and night. Daily vaginal smears were also made during this time. Representative females from all groups were then given an opportunity to breed with normal males for 2 weeks or more.

Several animals from each of the different groups were ovariectomized. The ovaries were dissected from the capsule, weighed, and at least one of each pair prepared for microscopic examination. A section of one uterine cornu was taken at the time of ovariectomy from representative individuals; whereas, other spayed females from each of the experimental groups were set aside, with uteri in situ. On the tenth post-operative day a biopsy was taken from the midportion of the left cornu of the latter animals, which were then injected with a single dose of 100 R.U. of estradiol benzoate.⁴ Seventy-

⁴ Estradiol benzoate was supplied through the courtesy of Dr. Erwin Schwenk of the Schering Corporation, Bloomfield, N. J.

two hours following the injection a biopsy was taken from the mid-portion of the right cornu. All three types of uterine tissues, i.e., those from intact animals, those taken after a 10-day period of involution following ovariectomy, and those taken after 10 days of involution and subsequent estrogen injection, were prepared for histologic study. Ovaries and uteri were fixed in Bouin's fluid and stained with hematoxylin and eosin. Sections of uteri were also stained with the Masson trichrome technique. The biopsy specimens were compared as to size in cross-sectional area, condition of the endometrial stroma and epithelium, and the number and condition of the glands. Cross-sectional area was computed in square millimeters by the formula $ab\pi$ (when a and b are semiaxes of an ellipse).

The reactivity of the mechanism responsible for estrous behavior was tested in representative ovariectomized rats by three series of injections with ovarian hormones. The first consisted of 10 R.U. of estradiol benzoate followed 48 hours later by 0.4 mg. of progesterone⁵ (Boling and Blandau, '39). A second series, given 1 week later to the same rats, consisted of 100 R.U. of estradiol benzoate followed in 48 hours by 0.4 mg. of progesterone. Another week elapsed before the third series was started, composed of four daily injections of 100 R.U. of estradiol benzoate followed on the fifth day by 0.4 mg. of progesterone. After each series the animals were examined at hourly intervals for 15 hours for display of the copulatory reflex.

Mammary glands were removed from three intact females that received 1.2 mg. of estradiol dipropionate, begun on the first day. These were fixed in Bouin's fluid, stained in hemalum, dissected free from adventitial fat and connective tissue, cleared in xylol, and mounted whole in gum damar. Similar preparations were made of mammary glands from untreated, intact females of the same age.

⁵ Progesterone was supplied through the courtesy of Dr. Erwin Schwenk of the Schering Corporation, Bloomfield, N. J.

RESULTS

Observations on the occurrence of cyclic manifestations of estrus in the adult animals, treated at various prepuberal ages with female sex hormone, are summarized in table 1. All doses begun on the first day and the dose of 1.2 mg. begun on the fifth and tenth days were equally effective in precluding estrous behavior. Unlike rats given androgen at corresponding ages (Wilson, Hamilton and Young, '41), these failed to give even aberrant behavioral responses. In the vaginal smear

TABLE 1

Occurrence of estrous cycles in adult females treated prepuberally with estrogen.

AGE AT BEGINNING OF TREATMENT	TOTAL DOSE	NUMBER OF ANIMALS	NUMBER OF ANIMALS DISPLAYING CYCLIC ESTRUS DURING A 12-DAY PERIOD OF OBSERVATION	
			Mating behavior	Vaginal smear
<i>days</i>	<i>mg.</i>			
1	0.037	4	0	1 (?)
	0.075	4	0	1 (?)
	0.15	4	0	0
	0.3 ¹	3	0	2 (?)
	0.6	9	0	0
	1.2	5	0	*
	2.4	4	0	*
5	1.2	5	0	0
10	1.2	5	0	3
15	1.2	4	2	4
20	1.2	7	7	7
30	1.2	6	6	Not observed
40	1.2	6	5	Not observed

* Vagina never opened.

leukocytes were the most numerous and consistently occurring type of cell. A few animals, however, that received the lower doses begun on the first day and three of five that received 1.2 mg. begun on the tenth day tended to show some fluctuation in the cell types. Such changes took place slowly over the course of several days—that is, from diestrous to estrous smear during a 6- to 10-day interval—and were thought to be of questionable cyclic nature. No animals in which treat-

ment was begun on the tenth day or earlier became pregnant when placed with normal males.

Estrous cycles were not abolished by estradiol dipropionate injections started on the fifteenth, twentieth, thirtieth and fortieth days (table 1). Twenty of twenty-three females displayed normal heat behavior at regular 4- or 5-day intervals, and two of the three which did not come into heat showed normal cyclic changes in the vaginal smear. The occurrence of heat always coincided with some degree of cornification in the vagina. Representative animals from each of these groups became pregnant and bore litters when placed with normal males.

TABLE 2

Ovarian weight and structure in adult females treated prepuberally with estrogen.

AGE AT BEGINNING OF TREATMENT	TOTAL DOSE	NUMBER OF ANIMALS	MEAN WEIGHT OF OVARIES	NUMBER WITH CORPORA LUTEA	CONDITION OF FOLLICLES
<i>days</i>	<i>mg.</i>		<i>mg.</i>		
1	0.037- 2.4	12	18.0	0	Small, some normal in appearance, many atretic
5	1.2	4	29.5	0	Small, some normal in appearance, many atretic
10	1.2	4	31.0	3	Mostly normal
15	1.2	4	58.3	4	Normal
20	1.2	4	68.5	4	Normal
30	1.2	4	66.0	4	Normal
40	1.2	4	55.5	4	Normal
Control		6	63.1	6	Normal

The eleven females that received pure peanut oil over the first 28 days of life were allowed to remain with their brother siblings. Since all of these bore litters by the ninety-eighth day, they were considered to possess normal reproductive capacity and were not subjected to further study.

Ovarian abnormalities were, in most cases, correlated with failure to exhibit cyclic estrus. Animals given all doses of estrogen, from 0.037 to 2.4 mg., beginning on the first day had exceedingly small ovaries, usually less than one-third the weight of those from control rats (table 2). Corpora lutea

were not present and the follicles were small, seldom exceeding the size attained by normal follicles in the early vesicular stage (fig. 1). Atretic follicles in all stages of regression were numerous. The inter-follicular spaces throughout the cortex were occupied by nests and cords of interstitial cells which possessed only scant cytoplasm and small, intensely-staining nuclei (figs. 2 and 3). These cells (fig. 4) resemble the so-called deficiency "wheel cells," described as appearing in the rat ovary after hypophysectomy and thought to signify a lack of hypophyseal hormones (Selye, Collip and Thomson, '33).

Ovaries from females treated beginning on the fifth day were somewhat larger than those from rats injected beginning on the first day; yet they were less than half the size of normal ovaries. The histologic picture was much the same as that described above, except that the interstitial tissue appeared less atrophic.

Although ovaries from rats treated first on the tenth day were consistently small, averaging considerably less in weight than the normal (table 2), a variety of structural conditions existed. One of the four pairs of ovaries showed no evidence of luteinization; but many large normal-appearing follicles were present. In a second there was recognizable lutein tissue, but this was scarce and did not appear to be of recent origin. The remaining two pairs, aside from their small size, gave every indication of being normal. Two or more different generations of corpora lutea could be distinguished on the basis of the criteria used by Boling ('42). The interstitial tissue in all of these ovaries was comparable in appearance to that in the ovaries of untreated rats; however, it was more abundant in cases in which corpus luteum formation was absent or deficient.

Ovaries from females given estrogen for the first time on the fifteenth, twentieth, thirtieth and fortieth days were normal with regard to size, corpus luteum formation and follicular development (table 2 and fig. 5).

The uteri of all rats treated prepuberally beginning on the first, fifth and tenth days showed some abnormalities; but

the severity of damage tended to diminish as the age at the time of initial treatment increased (table 3). Injections started on the first day produced the most extensive alterations (fig. 6). Size in terms of cross-sectional area was small. Glands were absent or scarce and, when present, appeared atrophic. In ten of twelve cases the endometrial epithelium had undergone squamous metaplasia, which condition varied in extent from one involving the entire lining to one affecting only scattered areas; and portions of the epithelium that had not suffered this change were atrophic. Transformation of

TABLE 3

Size and structure of uteri in intact rats treated prepuberally with estrogen.

AGE AT BEGINNING OF TREATMENT	TOTAL DOSE	NUMBER OF ANIMALS	MEAN CROSS- SECTIONAL AREA	MEAN NUMBER GLANDS PER SECTION	CONDITION OF EPITHELIUM		
					Metaplastic	Atrophic	Normal
<i>days</i>	<i>mg.</i>		<i>sq.mm.</i>				
1	0.037- 2.4	12	1.81	1	10	2	
5	1.2	2	1.66	2		1	1
10	1.2	2	2.52	4	1		1
15	1.2	2	3.38	4			2
20	1.2	2	3.39	5			2
30	1.2	2	4.04	7			2
40	1.2	2	3.43	5			2

this sort, after more or less prolonged injections of estrogens into mature rats, have been previously described (McEuen, '36; Zondek, '37; Korenchevsky and Hall, '40); but in the present instance the metaplasia was induced by treatment during the first postnatal month, at least 3 months prior to examination. The endometrial stroma was also altered: coarse connective tissue fibers had almost entirely replaced the cellular, loosely-knit reticulum characteristic of the rat uterus at this age (fig. 7). Nuclei were scarce, pyknotic, and spindle-shaped—much like those found in ordinary fibrous tissue. The myometrium was likewise fibrotic, the circular muscular layer usually more so than the longitudinal layer. That estrogenic treatment causes an increase in the connective tissue of the female genital tract has been repeatedly

shown (McEuen, Selye and Collip, '36; Korenchevsky and his co-workers, '39, '40; Burak, Wolfe and Wright, '42). Burak, Wolfe and Wright compare this change to that known to be associated with senility in the normal rat.

Uteri from animals treated beginning on the fifth and tenth days exhibited many of the above described alterations: small size, scarcity of glands, and fibrosis; but generally these conditions were somewhat less severe than in animals treated beginning on the first day (table 3).

The functional capacity of uteri from animals first injected on the fifteenth day or later cannot be questioned, since females from these groups bore normal litters. Histologic examination revealed that more fibrous tissue was present than in uteri of untreated animals of the same age; but no other abnormalities were seen (fig. 8).

After ovariectomy the uteri in females treated beginning on the first, fifth and tenth days underwent a limited degree of regression. The reduction in cross-sectional area 10 days after removal of the ovaries was 43%, 10% and 48% respectively, as compared with a reduction of 60% or more in uteri of animals treated beginning on the fifteenth day or later (compare tables 3 and 4). The histologic signs of regression in the affected uteri were inconspicuous, usually the only change being further atrophy of the epithelium that had not been altered by metaplasia.

Moreover, the damaged uteri were not normally responsive to estrogenic stimulation when, 10 days after ovariectomy, the animals were injected with a single dose of 100 R.U. of estradiol benzoate. The percentage increase in cross-sectional area of the uterus, taken 72 hours after the injection, was considerably less than that of unaffected uteri from animals treated prepuberally beginning on the fifteenth day and later or from untreated control animals (table 4). Likewise, the histologic response was deficient, and in no case was a typically estrous condition produced (fig. 9). No increase in the number or activity of the glands was apparent. Areas of metaplasia in the epithelium seemingly became thicker and the degree

of desquamation greater; although epithelium which had not undergone transformation appeared to react in normal fashion. The density of the fibrotic endometrial stroma was slightly lessened by edema. On the other hand, rats treated beginning on the fifteenth day, and later, possessed uteri that responded to estrogenic stimulation normally. The percentage increase in size often exceeded the average of that of uteri from eight untreated control animals; and the histologic picture was always identifiable as one of estrus or of proestrus.

TABLE 4

Responsiveness to estrogen of uteri in animals treated prepuberally with estrogen.

AGE AT BEGINNING OF TREATMENT	TOTAL DOSE	NUMBER OF ANIMALS	MEAN CROSS-SECTIONAL AREA OF UTERUS (SQ.MM.)		MEAN PERCENTAGE INCREASE
			10 days after ovariectomy	72 hours after 100 R.U. of estrogen	
<i>days</i>	<i>mg.</i>				
1	.6- 2.4	6	1.02	2.01	97
5	1.2	2	1.50	2.65	77
10	1.2	1	1.42	3.00	111
15	1.2	1	1.43	4.18	185
20	1.2	2	1.42	5.63	296
40	1.2	1	1.34	5.06	278
Spayed at birth	1.2	5	.73	2.23	205
Control, spayed as adult	...	8	1.74	4.94	184

In rats spayed at birth, prepuberal treatment with estrogen did not greatly impair the responsiveness of the uterus to subsequent stimulation with estrogen. The increase in cross-sectional area was within the range of that shown by the unaffected uteri (table 4). Before the stimulating injection was administered, these uteri, although extremely small and atrophic, contained fewer of the permanent alterations than those from intact rats treated at the same age. Of the five animals in this group, only one showed metaplasia of the endometrial epithelium and two showed what was considered an excessive amount of fibrous tissue in the stroma. These

observations suggest that at least a part of the damage to uterine tissues in intact rats treated at the earlier ages was due to uninterrupted action of endogenous estrogen, rather than to the direct effects of the prepuberally injected hormone. An interesting contrast may be drawn between these and the earlier observation (Wilson, Hamilton and Young, '41) that the presence of the ovaries was not associated with lowered responsiveness to estrogenic stimulation in animals treated with androgen shortly after birth.

It was pointed out above that the administration of estrogen during early prepuberal life precluded the display of estrous behavior in the adult rat. To determine whether this was due to an inadequate secretion of the ovarian hormones that condition the animal for sexual behavior or to an insensitivity of the mechanism responsible for such behavior, representative ovariectomized rats were injected with three different doses of estrus-inducing hormones. Animals given estrogen prepuberally beginning on the first, fifth and tenth days were now totally insensitive to the quantity of estrogen which induced mating behavior in untreated spayed females (table 5). A dose of 100 R.U., ten times the quantity routinely used for this purpose (Boling and Blandau, '39), was likewise ineffective; and when the dose was raised to 400 R.U., only three of seventeen responded. Ten of the insensitive rats were given increased quantities of progesterone (0.8 to 10 mg.), after 100 R.U. of estrogen, and were found to be equally refractory to increased dosage with this hormone.

Contrary to what was concluded regarding the insensitivity of the uterus to estrogenic stimulation, the lowered responsiveness of the estrous behavioral mechanism appeared to be independent of the presence of the ovaries. Six animals spayed at birth and treated beginning on the first day were also refractory (table 5).

Most of the females treated beginning on the fifteenth, twentieth, thirtieth and fortieth days responded normally: six of eight came into heat after the smallest dose (table 5). The failure of one animal in the fifteen-day group to

respond to any of the doses is of questionable significance, since a few such unresponsive females are known to occur in healthy colonies of rats (Boling, Blandau, Rundlett and Young, '41).

The mammary glands, from three females that received a total dose of 1.2 mg. of estradiol dipropionate beginning on the first day, differed in several respects from those of untreated rats (fig. 10) of comparable age. The mammary tree was more dense due to thickening of the ducts, to more

TABLE 5

Responsiveness of the estrous behavioral mechanism in spayed adult rats treated prepuberally with estrogen.

AGE AT BEGINNING OF TREATMENT	TOTAL DOSE	NUMBER OF ANIMALS	NUMBER DISPLAYING ESTROUS BEHAVIOR AFTER DIFFERENT DOSES OF ESTROGEN FOLLOWED BY 0.4 MG. OF PROGESTERONE		
			10 R.U.	100 R.U.	400 R.U.
<i>days</i>	<i>mg.</i>				
1	.037-	8	0	0	2
	.3				
	.6-	5	0	0	0
	2.4				
5	1.2	2	0	0	1
10	1.2	2	0	0	0
15	1.2	2	1	1	1
20	1.2	2	2	2	2
30	1.2	2	1	2	2
40	1.2	2	2	2	2
Spayed at birth	1.2	6	0	1	3
Control		10	9	10	10

profuse and irregular branching and to a considerable increase in the number and size of the end-buds (fig. 11). Indeed, the end-buds were often packed together in such clusters as to resemble the lobular structure of actively secreting glands; but there was no indication of true acini formation or actual secretion. In general this picture agrees with that described by Astwood, Geschickter and Rausch ('37) for glands from castrate females given 200 γ of estrin daily for 56 days beginning on the twenty-first day. These authors, however, reported extensive cystic changes, which were not

observed in my preparations. The differences in the duration of treatment as well as in the time at which the glands were removed, in the two instances, should be borne in mind. Although not examined microscopically, the glands of females treated beginning on the fifteenth day and later were functionally competent because such rats successfully nursed their litters.

DISCUSSION

The age of the animal at the time of treatment is of considerable importance in determining the extent of modification of reproductive capacity following prepuberal administration of estrogen. Injections begun on first day and, in general, those begun on the fifth day permanently altered all aspects of the sexual system that were studied. By the fifteenth day, however, the rats were no longer susceptible to the damaging effects of this type of treatment. The abruptness of the change is suggestive that it may possibly be associated with some more fundamental physiologic or morphologic change in the young animal; although at present there is no direct evidence to support such a conclusion. This hypothesis, nevertheless, receives some support from other observations (Wilson, Hamilton and Young, '41) in which the androgenic hormone, testosterone propionate, was also found to impair reproductive function in female rats when treatment was started on the first, fifth or tenth postnatal days; but, again, by the fifteenth day of life, treatment with this hormone left no persistent defects. The fact that the effectiveness of two hormones, of different biological activity, bears the same relationship to the age of the animal at the time of treatment, strongly indicates that a factor governing the animal's sensitivity to such hormones undergoes a spontaneous change at some time in early postnatal development. In this connection, it is interesting to recall Wiesner's ('34) report that the uterus and vagina of the immature rat do not attain full responsiveness to estrogenic stimulation until about the fifteenth day of life. Furthermore, Wilson and Young ('41) found that young female guinea pigs and rats could not be

induced to display sexual behavior earlier than the fifteenth or twentieth day, even when very large quantities of female sex hormones were injected.

The failure of the ovaries to form corpora lutea, in animals given estrogen shortly after birth, was probably secondary to a deficiency of pituitary hormone. Numerous other workers have attributed this ovarian defect to impairment of pituitary gonadotropic function, following the administration of estrogenic substances to rats during the latter part of the prepuberal period or during adult life (Leonard, Meyer and Hisaw, '31; Cramer and Horning, '36; Zondek, '36; Noble, '38 a; Korenchevsky and Hall, '40; and Selye and Friedman, '40). Neither in these reports on older animals nor in the present experiments, in which newborn females also were used, was there evidence of damage inflicted directly on the ovary by the injected substance. Spencer, D'Amour and Gustavson ('32 a) found that animals recovered spontaneously; ovarian cycles were resumed within 6 weeks after the cessation of estrin injections. Indeed, it has been shown that ovarian atrophy and failure of luteinization may be prevented by injecting gonadotropic substances concurrently with the estrogen, or that these abnormal conditions, even after prolonged estrogenic treatment, may be restored to normal by therapy with gonadotropins (Spencer, D'Amour and Gustavson, '32 b; Noble, '38 b; and Zondek, '41).

These observations indicate that estrogenic treatment of the adult or late prepuberal female produces no intrinsic damage in the ovary but causes severe impairment of pituitary gonadotropic activity, although normal function may be spontaneously resumed within a few weeks after treatment is stopped. The present results concurred with this, in so far as the injections initiated on the fifteenth, twentieth, thirtieth and fortieth days were concerned. Injections began on the first and fifth days after birth, however, resulted in a more persistent change in the pituitary gland. Six animals maintained for 5 months after the last injection showed no signs of recovery.

Pfeiffer ('36), Bradbury ('41) and Wilson, Hamilton and Young ('41) reported that female rats subjected to the action of androgen shortly after birth likewise suffered permanent gonadotropic deficiency. Corpora lutea were never formed in the ovaries. Pfeiffer ('42) was able to induce ovulation and luteinization in such ovaries, rendered acyclic by testis implants at birth, by injecting relatively pure preparations of the luteinizing hormone of sheep and swine pituitaries. Nevertheless, it cannot be inferred from these results that estrogen treatment at an early age suppresses pituitary gonadotropic function in the same way as does androgen treatment.

Further evidence of improper pituitary function was found in the ovaries of the estrogen-affected animals. The interstitial tissue was markedly atrophic: cytoplasm was scant and the nuclei pyknotic. In general appearance the cells resembled the deficiency cells with "wheel nuclei" described by Selye, Collip and Thomson ('33) in the ovaries of hypophysectomized rats. This is remarkably unlike the condition of the interstitial tissue in animals subjected during early life to the action of androgen, which has been frequently reported as being normal or even hypertrophied in appearance. Pfeiffer ('42), in accordance with the concept that the pituitary luteinizing and interstitial cell stimulating hormones are identical, has suggested that the interstitial cells respond to a lower level of the hormone than that required for luteinization. This view adequately explains the maintenance of interstitial tissue in the absence of luteinization, seen in ovaries of androgen-treated females, if it is assumed that the luteinizing hormone is present but at a diminished level. On this basis, it would appear that in the estrogen-affected females, possessing ovaries in which both interstitial tissue was atrophic and luteinization absent, the secretion of the luteinizing hormone had been entirely suppressed or reduced to an ineffectual level. The possibility that androgen and estrogen affect the pituitary gland differently during early life should be investigated further.

Sexual behavior was as completely abolished by the injection of estrogen during early postnatal life as it was previously reported to have been by similar treatment with androgen (Wilson, Hamilton and Young, '41). It was clearly demonstrated that, in both cases, the animals were not responsive to quantities of estrus-inducing hormones much in excess of those which cause heat in normal rats. At present it cannot be said whether this lack of responsiveness may be attributed to such derangements as: (1) destruction or elimination of the necessary hormones before the animal was properly conditioned, (2) insensitivity to the conditioning action of the hormones, or (3) interference with a neural effector system of sexual behavior. The latter possibility seems plausible in the light of some recent investigations in the field of neuro-physiology. Dempsey and Rioch ('39), Bard ('40), Brookhart, Dey and Ranson ('41), Dey ('41) and others have demonstrated the probable existence of a "center" for the control of sexual behavior somewhere in the hypothalamus or anterior mesencephalon. Lesions placed in these areas of the brain have been followed by permanent abolition of both spontaneous and induced sexual behavior. If such a center exists during early postnatal life, its structure or definitive function may conceivably be altered by an abnormally high level of sex hormone at this stage in development.

SUMMARY

Different groups of female albino rats were injected with estradiol dipropionate for a period of 28 days beginning at various prepuberal ages between birth and the fortieth day. Reproductive capacity in these animals was studied 3 or more months later.

The age of the animal at the time of treatment was of considerable importance. Treatment begun on the fifteenth, twentieth, thirtieth and fortieth days caused no permanent impairment of reproductive functions. The adult females displayed regular estrous cycles, mated, bore litters and lactated.

In contrast, when the injections were started on the first, fifth or tenth days, none of the adult females displayed cyclic manifestations of estrus nor did any mate with normal males. Treatment begun on the first day caused the most severe alterations. Spontaneous sexual behavior was never exhibited and the vaginal smear indicated a persistent diestrous condition. The ovaries were very small and contained no corpora lutea. Normal-appearing follicles were scarce and never progressed beyond the early vesicular stage, although numerous small atretic follicles were present. The interstitial tissue was extremely atrophic. Uteri were small, showed several pathologic changes and responded poorly to estrogenic stimulation. Estrous behavior could not be induced with much larger quantities of female sex hormones than were necessary to produce heat in untreated females. Mammary ducts were abnormally thickened and showed irregular and profuse branching.

Animals treated beginning on the fifth and tenth days also possessed many of these morphologic alterations, but usually to a lesser degree. Sexual behavior, however, was as completely abolished in these animals as in those injected beginning on the first day.

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PLATE 1

EXPLANATION OF FIGURES

1 Ovary containing numerous small and atretic follicles but no corpora lutea, from a rat affected by estradiol dipropionate injections (2.4 mg. total dose) begun on the first day. $\times 24$.

2 Ovary with excessive but atrophic interstitial tissue, from a rat affected by estradiol dipropionate injections (1.2 mg. total dose) begun on the first day. $\times 24$.

3 High magnification of figure 2 to show the extreme atrophy of interstitial tissue. $\times 430$.

4 Higher magnification of figure 3 to show "wheel nuclei" in atrophic interstitial cells. $\times 970$.

5 Ovary containing large normal follicles and two generations of corpora lutea, from a rat unaffected by estradiol dipropionate injections (1.2 mg. total dose) begun on the fifteenth day. $\times 24$.

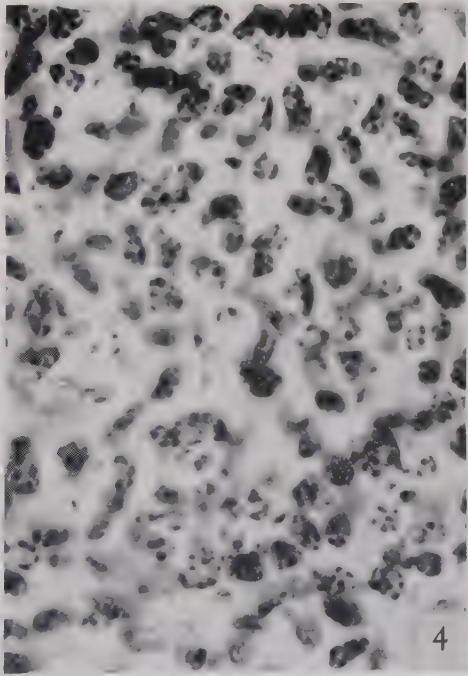
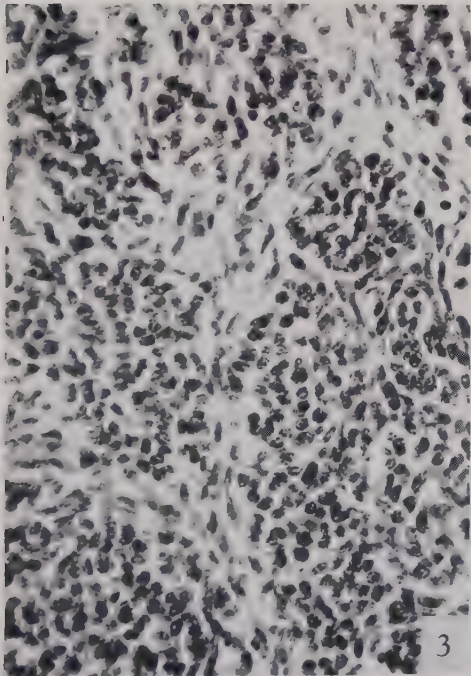
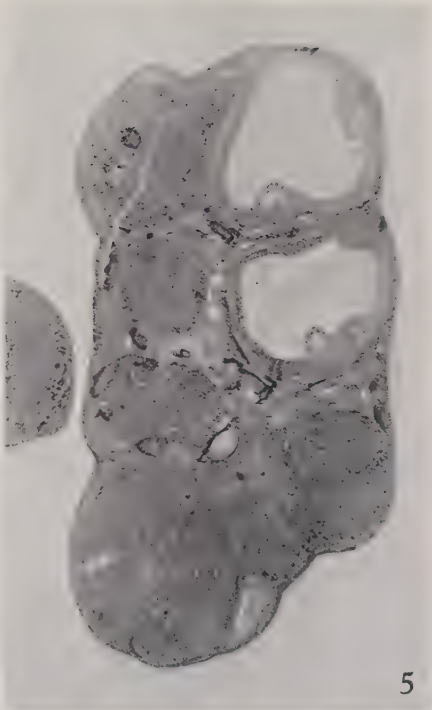
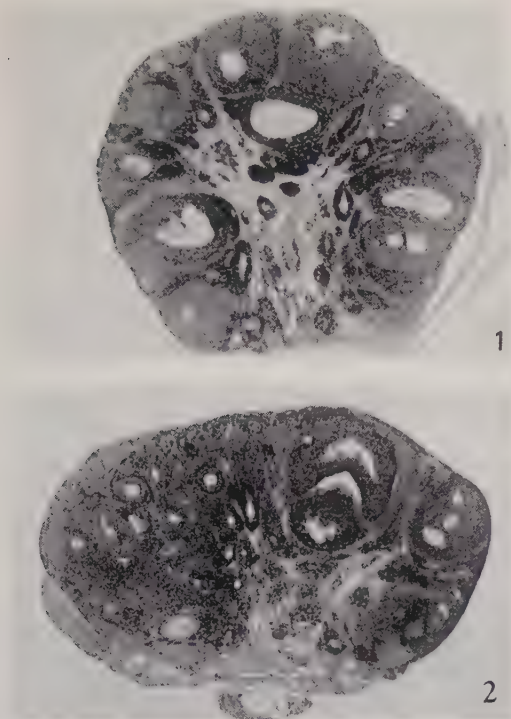


PLATE 2

EXPLANATION OF FIGURES

6 Uterus showing metaplasia of epithelium, scarcity of glands, and fibrosis of endometrial stroma, from an intact rat affected by estradiol dipropionate injections (0.6 mg. total dose) started on the first day. $\times 24$.

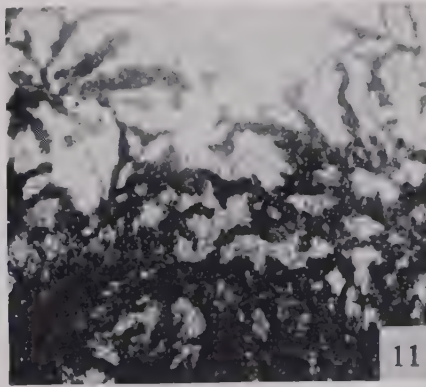
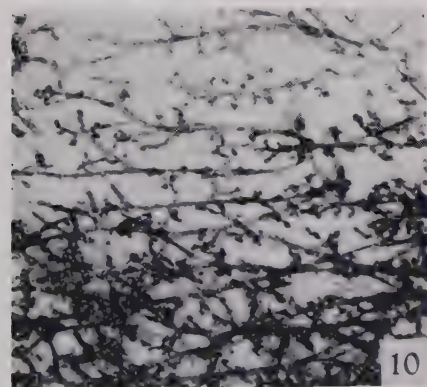
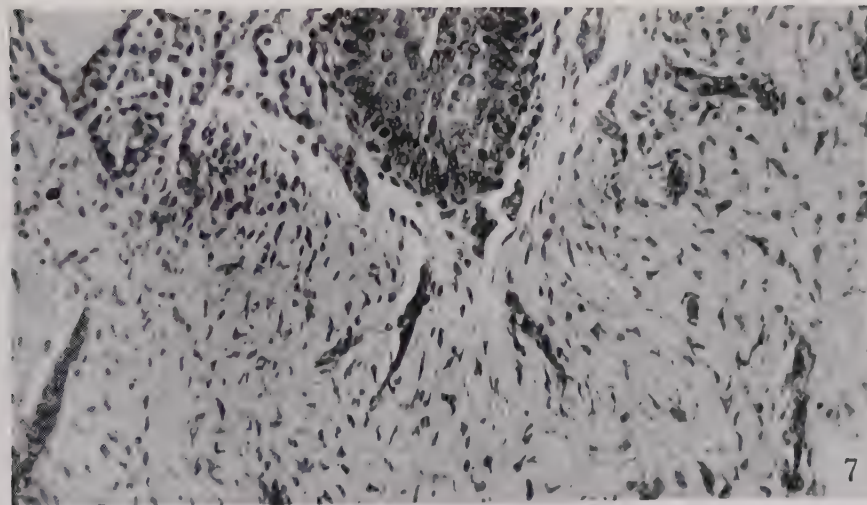
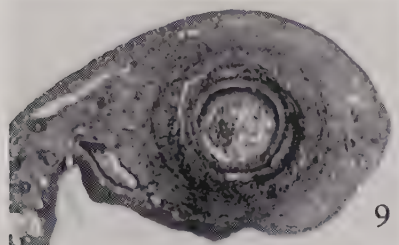
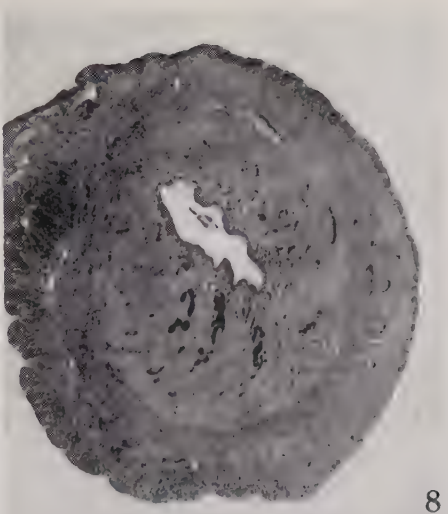
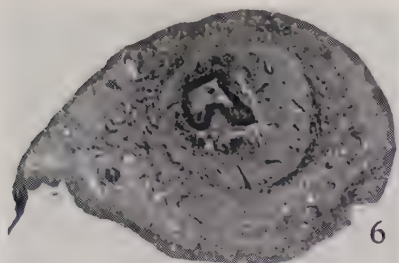
7 High magnification of figure 6 to illustrate epithelial metaplasia and endometrial fibrosis. $\times 270$.

8 Uterus corresponding to the normal diestrous condition, from a rat unaffected by estradiol dipropionate injections (1.2 mg. total dose) begun on the twentieth day. $\times 24$.

9 Uterus taken 72 hours after injection of 100 R.U. of estradiol benzoate to show failure of the normal response to estrogenic stimulation, from a spayed rat affected by estradiol dipropionate injections (0.6 mg. total dose) begun on the first day. $\times 24$.

10 Portion of whole mount of a mammary gland from an untreated female rat. $\times 11$.

11 Portion of whole mount of a mammary gland, showing thickened and irregularly branched ducts, from a female affected by estradiol dipropionate injections (1.2 mg. total dose) begun on the first day. $\times 11$.



SEX AND SEASON IN RELATION TO MALFORMATIONS OF CHICKEN EMBRYOS

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An appreciable percentage of chicks which fail to hatch show morphological abnormalities. The majority of these malformed embryos fall into a few well-defined groups. Little is known concerning the sex incidence among such deformed embryos. It seemed worthwhile, therefore, to put on record some observations which we had an opportunity to make on material from sex-linked crosses in which the sex could be distinguished by the color markings of the down.

MATERIAL

We used for our observations the unhatched eggs from matings of Barred Plymouth Rock females by Rhode Island Red males. These unhatched eggs were supplied, between October 1941 and May 1942, by a large commercial hatchery.

Upon receipt the eggs were opened and the embryos examined for gross abnormalities. The "normal" embryos of the first few shipments were discarded without sexing, but in all later shipments the sex of all embryos was recorded. All deformed embryos were preserved in formalin for subsequent classification and sexing. It should be emphasized that no search was made for internal malformations, that variations of the rump were not included in the abnormal group, and that embryos which had died at relatively early stages frequently had disintegrated too much to be examined. It follows that our groups of "normal" embryos probably included appreciable numbers of specimens with internal morphological deformities and that for all types of malfor-

mations our figures gave a reasonably accurate picture only with regard to those which survived into the last week of incubation.

In many cases of exencephaly the sex of the embryos could not be determined from the color pattern of the down, but had to be ascertained by dissection.

SEX AND MALFORMATIONS

A summary of all sex records is given in table 1. It can be seen that among 8113 embryos without gross external deformities there were 3611 or $44.5 \pm 0.56\%$ males. This is a significant deviation from equality, indicating a greater mortality of "normal" females prior to hatching. Very

TABLE 1

Totals of all "normal" and malformed embryos which were examined and the frequencies of males among them.

DATE	EMBRYOS WITHOUT GROSS DEFECTS ("NORMAL")			MALFORMED EMBRYOS		
	Males			Males		
	Total	Actual	Per cent	Total	Actual	Per cent
10/ 8-10/29		...		70	33	47.1
11/ 4-11/11		...		100	58	58.0
11/25-12/ 2	1194	533	44.6	100	54	54.0
12/ 9- 2/11	1926	890	46.2	114	50	43.9
2/26- 3/ 6	1303	594	45.6	164	93	56.7
3/26- 4/ 6	1157	526	45.5	81	35	43.2
4/ 7- 4/17	1575	678	43.0	182	83	45.6
4/25- 5/ 2	958	390	40.7	98	42	42.9
Total	8113	3611	44.5 ± 0.56	909	448	49.3 ± 1.67

similar figures are obtained if the defective embryos are included in the total. In this case it is found that (beginning with the data of November 25th) 3968 or $44.7 \pm 0.52\%$ of the 8872 embryos were males. These figures are in good agreement with data published by Byerly and Jull ('35).

Among the hatched chicks of our material about 51% were males and approximately 80% of all eggs hatched. Taken together these figures agree rather well with the conclusion that the primary sex ratio was normal.

Among the 8872 embryos 739 or $8.3 \pm 0.29\%$ were classified as abnormal and out of these 739 malformed embryos 448 or $49.3 \pm 1.67\%$ were males. The percentage of males is somewhat higher among the deformed embryos than among the normal ones, but this difference is of no particular interest since subsequent data will demonstrate that various types of malformations behave differently with regard to their sex incidence.

The frequency with which the two sexes occurred among either normal or abnormal embryos did not indicate the existence of seasonal trends in the sex ratio.

The numbers and percentages of males according to the different types of malformations found in our material are given in table 2. Some of the groups contain too few individuals to justify any conclusions. In other groups the two sexes evidently occur with approximately equal frequency. This is true for micro- and anophthalmia (49.1% males among a total of 281 cases), exencephaly and hyperencephaly. Hutt and Greenwood ('29) also failed to find any sex differences in the incidence of these malformations.

In at least one type of malformation, however, the sex ratio deviates very markedly from equality or, more correctly, from the 44.5% of males which occurred in the entire material of unhatched embryos. Among fifty-one cases of otocephaly only twelve or $23.5 \pm 6.0\%$ were males. It is evident that there is a significant excess of females among otocephalic chicken embryos.

Two other groups call for comment. A deficiency of males seems to occur among duplications associated with exencephaly. In a group of thirty-eight embryos with posterior duplication and exencephaly fifteen or 39.5% were males; among thirty-nine embryos with duplication of the facial parts and exencephaly fifteen or 38.5% were males; and in an additional group of embryos with posterior duplication and exencephaly (collected 2 years earlier from the same source) eight out of twenty-three or 34.8% were males. Thus, among a total of 100 embryos with duplication and exencephaly only

thirty-eight were males. It is true that this deviation is not statistically significant, but the consistently low frequency of males in three different groups indicates the desirability of collecting further data. This is particularly true in view

TABLE 2
Incidence of males among the different types of malformations.

TYPE OF MALFORMATION	TOTAL	MALES	MALES <i>per cent</i>
Micro- or anophthalmia left, cross-beak	106	57	53.8
Micro- or anophthalmia right, cross-beak	88	37	42.0
Micro- or anophthalmia bilateral, with or without short upper and/or cross-beak	87	44	50.1
Exencephaly, generally with short upper beak	158	80	50.6
Hyperencephaly	38	19	50.0
Otocephaly	51	12	23.5
Short upper (or, more rarely, lower) beak	38	15	39.5
Cross-beak	24	13	54.2
Other beak abnormalities.	5	2	40.0
Micromelia	124	76	61.3
Left leg or foot abnormal	8	4	50.0
Right leg or foot abnormal	5	4	80.0
Both legs and feet abnormal	4	4	100.0
Short spine	5	3	60.0
Ectopia viscerum	2	1	50.0
Posterior duplication	36	17	47.2
Posterior duplication with exencephaly and short upper beak	38	15	39.5
Anterior duplication	2	1	50.0
Duplication of face with exencephaly	39	15	38.5
Duplication of upper beak	4	1	25.0
Schizosoma reflexum ¹	1	0	0
Unclassified	46	28	60.9
Total	909	448	49.3

¹ To the writer's knowledge the first case of this malformation recorded for birds.

of the fact that in man duplications are known to occur more rarely in males than in females (Schwalbe, '07).

There is a curious excess of males in the group of embryos with abnormal legs or feet, but the totals are much too small for a decision as to its significance.

SEASONAL TRENDS IN FREQUENCY OF VARIOUS MALFORMATIONS

The majority of authors who have reported on sporadic chondrodystrophy in chicken embryos are agreed that this abnormality is most commonly found during winter and that its incidence decreases with the advance of spring. Since it is impossible from gross inspection alone to be sure of the diagnosis "chondrodystrophy," all cases of disproportionate shortening of the extremities have here been classed as micromelia. With regard to seasonal incidence of micromelic embryos our present data confirm those of earlier observers.

Among 384 deformed embryos collected between October 8th and February 11th, there were sixty-six or 17.2% with micromelia, whereas 525 deformed embryos collected between February 26th and May 2nd, included only thirty-four or 6.5% micromelic specimens.

Sporadic rumplessness, on the other hand, was found to occur more commonly in summer than in the earlier part of the hatching season (Landauer and Baumann, '43). A similar trend, i.e., increasing incidence from mid-winter to the beginning of summer, was reported by Upp ('34) for eye abnormalities of chicken embryos and our data on micro- and anophthalmia are in fair agreement with Upp's observations.

The frequency of duplications appears to be influenced also by seasonal factors. If all types of duplications in our material are treated as one group, it is found that the incidence rises more or less regularly throughout the period during which observations were made; duplications were rarest in the hatches of October and early November and most common in those of late April and early May. The coefficient of correlation between time of hatch and number of duplications among the unhatched chicks equalled $+0.953 \pm 0.035$ and is highly significant.

Other abnormalities (exencephaly, hyperencephaly, otocephaly) showed no apparent seasonal trend of incidence.

DISCUSSION

A great many data on apparent deviations from a normal sex ratio have been recorded in the literature, especially in that dealing with human pathology. In some cases, e.g. albinism, it is evident that the seeming differences find their explanation in the lesser likelihood that females come to the attention of the observer (Haldane, '37-'38). In other instances there is no doubt that sex differences in incidence actually do exist and for some of these, e.g. harelip and the Laurence-Moon-Biedl syndrome, sex-linkage can be excluded as an explanation (Csik and Mather, '37-'38).

In our material one type of malformation shows a clearly significant deviation from a normal sex-ratio, viz., otocephaly. Female embryos with otocephaly are about three times as common as are male embryos with the same defect. This is of considerable interest in view of the fact that a very similar situation exists in guinea pigs (Wright, '34), female otocephalics in these animals being about twice as common as males. According to Wright a preponderance of females probably exists also in otocephaly of pigs and man.

Furthermore, Murphy ('39) has shown that the same holds true for human cases of the related condition of anencephaly.

Parallels to human pathology exist also for the two instances in which aberrant sex incidence is suggested rather than proven by our data. There is a prevalence of females among chicken embryos with duplication associated with exencephaly and among human double-monsters females are definitely more common than males (Schwalbe, '07). On the other hand, a predominance of males is suggested for our material on skeletal defects of the extremities, and the same seems to hold for many long bone defects in man, such as defects of femur, tibia, fibula, ulna and radius.

Otocephaly of guinea pigs has a definite, though complex, genetic basis. In chickens, on the other hand, no hereditary factors are known to be responsible for the occurrence of otocephaly. In fact, most of the malformations which in the

present material occurred in appreciable numbers are found with similar frequencies in stocks of widely different origin and composition and in cross-bred or inbred matings. These defects are, therefore, likely to be due to developmental accidents rather than to hereditary transmission.

In discussing the excess of females in his otocephalic guinea pig material Wright came to the conclusion "that the X chromosome is effective as a primary sex determining factor perhaps through an effect on rate of metabolism near the beginning of development, although differential absorption at a later period is a possible alternative." These two possibilities must also be considered with regard to our material of otocephalic chicken embryos. If the explanation for the excess of females were to be found in greater morbidity and earlier mortality of male embryos, then one should expect the average age of the male otocephalics in our material to have been less than that of the females with otocephaly. This was not the case. Hence, it seems much more likely that sex itself plays a role in the etiology of otocephaly. Since, however, the sex chromosome mechanism of birds is the opposite to that of mammals, it must be assumed that it is not homozygosity for the X chromosome which increases the chances for the expression of otocephaly, but physiological processes which are associated with female sex determination.

With regard to seasonal factors which influence the frequency of various types of deformities, it is obvious that different causative agents must be operative. In the case of micromelia (chondrodystrophy) it seems most likely, as has been suggested earlier, that physiological changes which act on the laying females are responsible for the decline of this abnormality with the approach of summer. In other instances (rumplessness, eye abnormalities) it seems probable that with increasing temperatures of the surroundings the blastoderms become more likely to reach, between laying and the beginning of incubation, critical stages in which their susceptibility to disturbances is heightened. Neither of the foregoing alternatives readily explains the steady increase,

throughout the laying year, in the number of duplications, and it seems reasonable to assume that still other agencies (age of mother?) influence the chances that certain malformations will occur.

SUMMARY

A study of chicken embryos which had died during the second half of the incubation period revealed the following facts:

Among embryos with otocephaly females are much more common than males.

The frequency of embryos with micro- or anophthalmia rose from mid-winter toward early summer.

The incidence of embryos with various types of duplications increased throughout the laying year of the mothers (September to May).

ACKNOWLEDGMENT

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GROSS VARIATIONS IN THE ILEOCECAL VALVE A STUDY OF THE FACTORS UNDERLYING INCOMPETENCY ¹

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TWO PLATES (SIX FIGURES)

For many years the interest of this surgical clinic has been devoted to the problem of intestinal obstruction (Wangenstein, '37) and to the mechanical features of special obstructions (Wangenstein, '36) as obtained in the closed-loop obstruction of the appendix (Wangenstein, Buirge, Dennis and Ritchie, '37) and colon (Sperling, '36). In acute mechanical obstruction of the colon in man the ileocecal valve and sphincter behave like a check-valve and prevent regurgitation of colonic contents into the ileum. However there are unpredictable instances of acute colonic obstruction (Buirge, '41) in which the ileocecal valve allows regurgitation to occur into the small intestine. The use of intestinal intubation by the Wangenstein method ('31, '32), or the long intestinal tube of Miller and Abbott and Johnston ('38) does not regularly produce relief in cases of obstruction of the colon due to the inconstant behavior of the ileocecal valve or sphincter.

At the suggestion of Dr. Owen H. Wangenstein (Rea, Smith and Schwyzer, '38-'39) that a study of the gross anatomic variations of the cecal topography of the ileocecal valve would be of interest in determining the factors responsible for competency of this junction, some 600 specimens of the cecum and terminal ileum were examined (Wangen-

¹ The researches presented herewith were aided by a grant of the Graduate School of the University of Minnesota and the drawings by a grant of the Work Projects Administration, Official Project No. 165-1-71-124, Subproject No. 399.

steen, '42). The material obtained was from cadavers at varying intervals after death. For purposes of orientation a number of specimens were dried with air, some were filled with melted Wood's metal, many were filled with gelatine and hardened in formalin solution then cut through various planes to study the interrelation of the parts of the valve. The following method was adopted in the treatment of some 500 fresh specimens. After cleansing the lumen of the ileum, cecum and ascending colon with water the specimen was filled with 10% formalin solution (both ends of the intestine being tied). It was then suspended in a vat containing similar fluid. This method of fixation it was felt eliminated contraction phenomena to some extent (Reider, '36). Specimens which had been injured or exhibited unusual contraction were excluded from the study.

OBSERVATIONS

The ileocecal eminence. That portion of the terminal ileum which has passed through the cecal wall into the cecal cavity and terminates in the formation of the ileocecal orifice is designated the ileocecal valve or sphincter. It is surrounded by a superior and inferior fold of the cecal wall which coalesce at both ends of the orifice to form the frenulum of the cecum. The segments of the lateral (right) and medial (left) frenula which divide and are reflected upon the lips of the valve are designated the "arches" of the frenula (fig. 1). The entire structure is referred to as the ileocecal eminence.

Measurements were made with calipers through the inferior and superior lips along their presenting surface. The thickness of the lips ranged from 0.3 cm. to 3.0 cm. The shape of the eminence varied from the classical flat, thin-lipped protuberance to a rounded, thick projecting mass of variable size, the summit often presenting a small or large opening into the terminal ileum.

The valvular aperture. The opening into the ileum was not constant as to size or contour. Oval, round and narrow transverse openings into the terminal ileum were encountered,

no correlation could be established between the size or shape of the orifice and the type of valve. Olive tipped metal sounds were gently passed into the ileum of 426 valves to determine the approximate size of the orifice. About 30% of the openings exceeded 1.5 cm. in diameter, while orifices 2.5 to 3.5 cm. in diameter were frequently encountered. In such large valves it appeared that combined sphincteric and mechanical valve action could only with difficulty maintain competency of the orifice. If the closure of the opening is mechanical or sphincteric, neither would be necessary to maintain effectively the competency of the valve in instances where the openings are less than 3 mm. in diameter (5%). Rutherford's case ('14) may have been an example of such an orifice.

The superior lip. The contour of the superior lip was generally convex and varied in thickness from 0.3 to 2.5 cm. It projected into the cecal lumen from 0.4 to 2.5 cm. (fig. 3 b). The average length of the projection in 443 valves was 1.5 cm. In 411 of 443 valves measured the superior lip extended beyond the inferior lip (Z—fig. 5 b). In 40% of the type I valves the superior lip projected 0.1 to 0.8 cm. beyond the inferior lip; frequently the projection exceeded 2.0 cm. in length.

The inferior lip. The inferior lip was slightly concave to semi-circular in shape, with the concavity directed toward the superior lip. The thickness of this segment was 0.3 to 1.6 cm., and it projected an average of 0.6 cm. from the cecal wall (Y—fig. 5 b). In three instances the inferior lip was absent as a projection into the cecal cavity, while the superior lip was completely developed.

The inferior lip was deficient or incompletely developed in 75% of 411 valves (Z—fig. 5 b) measuring 0.4 to 2.0 cm. less in length than the superior lip.

The frenula. When the cecum was filled with formalin solution and gradually distended, the frenula² were subjected to

² Observations and graphic records of the activity of the ascending colon, ileocecal valve and the terminal ileum were made on an unanesthetized human subject. The frenula valvuli coli were present and easily observed, and at no time was contraction of the frenula noted. The detailed observations upon the sphincter will be reported at another time.

stretching and tension, affording an artificial yet dynamic action upon the structures of the valve. The size and form of the ileocecal eminence did not influence the presence or absence of the frenula, as large and small, rounded, oval, and flattened forms were found, with many variations of the frenula which appeared fundamental to the structure and function of the ileocecal junction.

Hunter ('34) believed that the ceco-colic sphincter of the lower mammals is situated a variable distance above the ileocecal sphincter, while in man the ceco-colic sphincter is found at the level of the ileocecal junction, and there forms the frenula coli in the position described by Rutherford ('14) who found definite bands of muscle fibers present in the frenula and recognized the frenula as distinct structures of the ileocecal eminence.

For the purpose of orderly arrangement of the anatomical variations of the frenula it was convenient to separate the material into groups or types of valvular construction. In the classical or common type of valve (fig. 1) the medial frenulum divides into a superior "arch" and an inferior "arch" as it passes around the summit of the terminal ileum. In similar fashion the lateral frenulum separates to form the inferior and superior lateral "arches" which enfold the terminal ileum. The basis for grouping the variations depends upon the presence or absence of the arch from the frenulum, which is reflected upon the ileocecal summit.

TYPES OF VALVES

Type I (valve) (figs. 1 and 2). The classical type of ileocecal valve was seen in 54% of 500 specimens, however, the slit-like orifice so frequently illustrated in the literature was present in only one-third of the specimens possessing this type of valve. Both lips projected an equal distance into the cecum in 7% of the valves in this group, whereas in nearly 20% the inferior lip was 1.0 cm. or more shorter than the superior lip. Approximately one-third of the apertures of the terminal ileum exceeded 2.5 cm. in diameter. Wakefield and

Friedell ('41) and Wesson ('37) estimated that 50% of their seventy-five specimens had competent valves or the classical type of valve structure. Struthers (1893) described twenty-three specimens in detail and noted similar variations in the components of the valve; eleven of the twenty-three valves presented imperfectly developed frenula, and in such instances incompetency was either ascertained or manifest.

Type II (valve). Characterized by absence of the superior or inferior arch of either the medial or lateral frenulum (13%). The superior and inferior lips unite at both ends of the orifice to engage the frenulum of the cecum (type I), however many specimens were encountered in which the superior arch or inferior arch of the frenulum was incompletely developed or absent.

A. Absence of lateral inferior arch of the frenulum (fig. 3 a). In fifty-six specimens the lateral inferior arch was absent, resulting in an incompletely developed lateral frenulum of the cecum.

B. Absence of medial inferior arch of the frenulum (fig. 4). Eight such valves were encountered in this series. The medial frenulum was incomplete and consisted of a single fold from the superior lip.

C. Incomplete arches of the frenulum. Two specimens had defects of the valve involving absence of the superior arch of the lateral frenulum and the inferior arch of the medial frenulum.

While other possible defects are obvious, none were encountered.

Type III (valve). Bilateral absence of the inferior arches of the frenula (6.4%). The lateral and medial portions of the inferior lip approach the extremities of the superior lip in perpendicular fashion and unite as though the inferior lip were suspended from the superior lip (fig. 5 a). The lateral and medial inferior arches were not developed and appeared as though the frenula did not divide to surround the apex of the terminal ileum.

Type IV (valve). Absence of the lateral frenulum or the medial frenulum.

A. Absence of the medial frenulum (22%). The summit of the terminal ileum was circumferentially complete; however the medial frenulum was not present, while the lateral frenulum had appeared. This type of valve (fig. 6) is commonly described in the anatomical literature. Keith ('03) presented a detailed gross anatomic description of the disposition of the muscle fibers in such a valve, and Rutherford ('41) described the microscopic findings as observed in the frenulum of a specimen with this type of structure, although no acknowledgment was made by either author of this variation as a departure from the more classical configuration of the valve.

B. Absence of the lateral frenulum (2%). The medial frenulum was complete and engaged circumferentially the medial cecal wall, blending into the mucosal folds of the cecum. The lateral half of the superior lip ended abruptly to unite with the corresponding part of the inferior lip to form the lateral half of the valve which was not supported by the presence of the lateral frenulum.

Type V (valve). Absence of the lateral frenulum and the medial frenulum (2%). This valve was characterized by the absence of the frenula, the superior and inferior lips were not supported by either a lateral or medial frenulum. The valvula coli apparently thrusts itself into the cecal lumen without regard to the presence or absence of the frenula of the cecum.

COMMENTS

The variations of the ileocecal valve consist of alterations in the size and shape of the ileocecal eminence, the size and shape of the orifice, the absence of one or both frenula, the incompleteness of the frenula as indicated by the absence of one or more arches of the frenula, defects in the length, and variable thickness of the superior and inferior lips. There are other anatomical factors which very likely affect the competency of the valve or sphincter as illustrated in six specimens such as the presence of a pedunculated polyp of the

superior lip (1.0 to 2.0 cm. in diameter) harbored within the orifice of the terminal ileum.

If one assumes that a valve or sphincter is present at the ileocecal junction it is difficult to explain the modification in the degree of competency of the valve until the variations in the valvular structure or other anomaly has been ascertained. Further anatomical and clinical observations are needed before correlations of structure and function are better understood.

Recently a 60-year old man was operated upon at the University Hospitals by Dr. Clarence Dennis for a possible strangulation obstruction of the small intestine. There were no pre-operative signs or symptoms to suggest the presence of a carcinoma of the transverse colon. Examination of the ileocecal valve after a right hemicolectomy showed it to be a type II (A) valve and the cecum was a Treves' type III. The valve was completely incompetent.

More direct evidence is required to establish the factors underlying competency of the ileocecal valve.

CONCLUSIONS

1. The classical type of the ileocecal valve was observed in 54% of 500 post mortem specimens.
2. Anatomical variations of the ileocecal junction are classified according to observed deficiencies of the frenula valvuli coli.
3. It is evident that sufficiency or insufficiency of the ileocecal valve or sphincter may depend on its structure. A case is reported in which incompetency of the ileocecal junction was associated with a type III cecum and a type II (A) valve.

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PLATES

ABBREVIATIONS

S L, Superior lip	L F, Lateral frenulum
I L, Inferior lip	S L A, Superior arch of the lateral frenulum
M F, Medial frenulum	I L A, Inferior arch of the lateral frenulum
S M A, Superior arch of the medial frenulum	Abs., Absence
I M A, Inferior arch of the medial frenulum	

PLATE 1

EXPLANATION OF FIGURES

1 Classical type I valve. The terminal ileum projects as the summit of the small intestine, carrying with it symmetrical folds of the cecal wall, the medial and lateral frenula.

2 Type I valve. Variation of the superior lateral arch as it separates from the inferior lateral arch of the lateral frenulum. Both arches blend into the mucosa of the cecal wall. The medial frenulum shows the usual configuration.

3 a Type II (A) valve. Absence of the inferior lateral arch, resulting in an incomplete lateral frenulum of the valve.

3 b Vertical section through type II (B) valve. X — Projection of the superior lip from the cecal wall to apex of the orifice. Y — Projection of inferior lip from cecal wall to orifice. Z — Point of reflection of the superior medial arch of the frenulum; absence of the inferior medial arch of the frenulum. Th — Thickness of the superior lip as measured at the margin of the orifice.

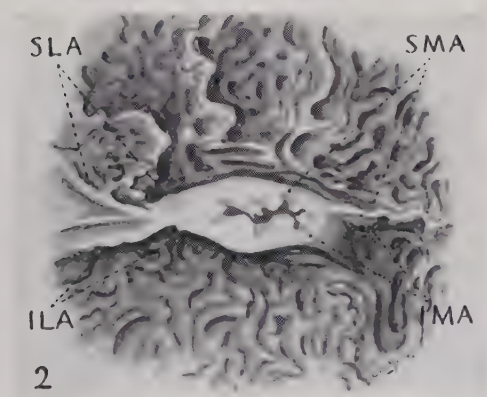
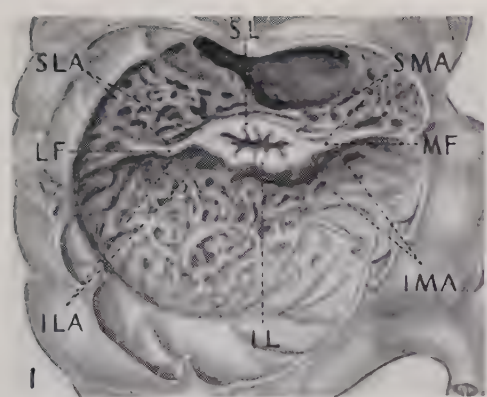


PLATE 2

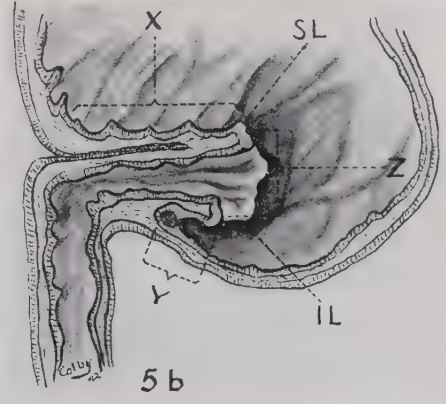
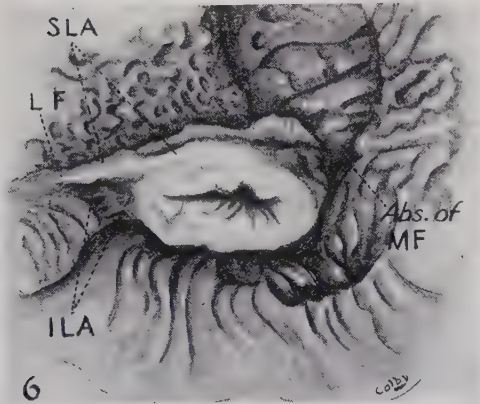
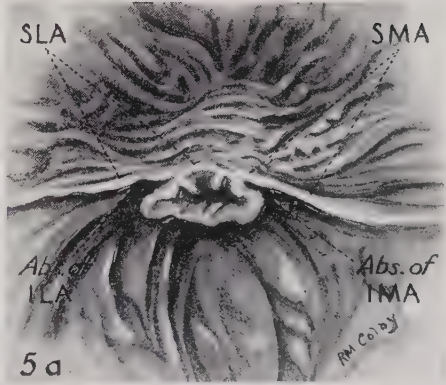
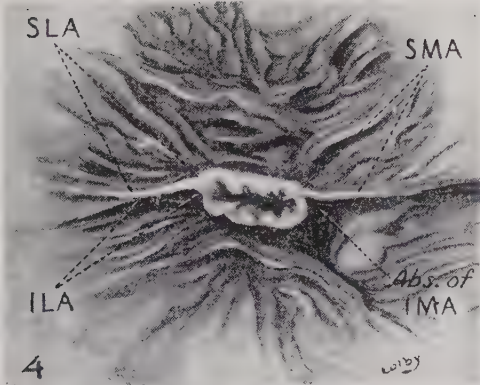
EXPLANATION OF FIGURES

4 Type II (B) valve. Incomplete medial frenulum due to absence of its inferior arch.

5 a Type III valve. Incomplete medial and lateral frenula due to absence of both inferior arches. (There is considerable redundancy of the inferior lip.)

5 b Vertical section type III valve. X — length of superior lip from cecal wall to apex of orifice. Y — Short inferior lip. Z — Difference in length of the superior and inferior lips as observed at the apex of the orifice.

6 Type IV (A) valve. Absence of the medial frenulum.



SOME CURIOUS EFFECTS OF SALTS OF METALS AND OTHER CHEMICALS ON FIXATION

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ONE PLATE (SEVEN FIGURES)

Ten years ago I published in *Science* several formulae of fixing fluids containing phenol or its nitro derivatives. My intention was to provide a fixing fluid for general use, which would give satisfactory fixation and at the same time leave the tissues as soft as possible. The cupric paranitrophenol fluid no. 2 has been used extensively by myself and other investigators since the publication of its formula. Prolonged experience showed that fixation of mammalian and invertebrate material is fairly satisfactory, but that the fluid causes peculiar changes particularly conspicuous in the nuclei of amphibian tissues. Addition of formalin to the fixing fluid at the time of use slightly improves fixation, but does not give pictures comparable with such obtained after fixation in fluids regularly used by cytologists. Determined to eliminate this distorting effect I continued my investigations with a long series of salts of heavy metals and other ingredients. In the course of these investigations the cause of the distorting effect was traced to its origin and finally successfully eliminated. Once found, the solution proved to be so simple that much time and labor could have been saved had it occurred to me by some lucky chance at the beginning and not at the end of the work. However, some additional observations were made, showing other undesirable effects produced by ingredients which successfully counteract the distortion of nuclear structures in amphibia. It seems therefore desirable to give

a more or less detailed account of the investigation and thus put on guard other investigators who might be misled in the interpretation of certain pictures due entirely to peculiarities of fixing fluids.

My selection of salts and compounds of metals was governed by a desire to get as complete precipitation of proteins as possible. It is well known that precipitating agents of proteins vary a great deal in this respect and that some proteins, even when precipitated, may be again redissolved and thus washed out of the cells. Experience has also shown that the presence of other ingredients may make precipitation of proteins by metallic salts more complete and permanent. Zirkle called attention to differences in fixation produced by salts in which the metal in solution forms a part of the cation, and such in which it forms a part of the anion. As will be seen from an examination of the following list, I experimented with both types. I also tried such salts in which one metal forms part of the cation and another metal part of the anion. Naturally, the selection was also governed by the solubility of the salts in water and alcohol, by the stability of the solution and to some extent by the cost and the possibility of purchasing the chemicals in pure form. The sequence in which they are listed is simply an alphabetic one and has nothing to do either with their importance or with the progress of the work.

Antimony: antimony trichloride, antimony trifluoride.

Chromium: chromic acid, chromic ammonium sulphate, chromic potassium sulphate, chromic sulphate, potassium bichromate.

Cobalt: cobaltous nitrate.

Copper: cupric acetate, cupric bromide, cupric chloride, cupric chromate, cupric bichromate, cupric nitrate, cupric sulphate, cupric potassium cyanide, cuprous potassium cyanide.

Mercury: mercuric acetate, mercuric bromide, mercuric chloride.

Thallium: thallous carbonate, thallous sulphate.

Uranium: uranyl nitrate.

Salts of iron, lead, silver, gold and platinum were tried only in a few combinations with phenol derivatives.

The phenomenon of nuclear distortion after fixation in the paranitrophenol-cupric fixing fluid consists in the following: the chromatin condenses at the end of the nuclei opposite to the exposed surface of the tissue. Chromatin threads can be scarcely discerned. The mass of chromatin is so compact that in preparations stained with haematoxylin-eosin it appears deep blue, almost black, while the rest of the nucleus remains quite transparent owing to the total lack of chromatin in that region. Gradual elimination of the various ingredients of the paranitrophenol-cupric fixing fluid led to the recognition of the totally unexpected fact that cupric nitrate alone is responsible for the peculiar appearance of the nuclei. Omission of the nitric acid increases the effect. Omission of the alcohol, ether and paranitrophenol does not alter the effect. Other salts of copper were then tried out and gave the same result. The experiment was extended over the entire list given above: some of the salts produced the effect, others did not. The answer to the question as to the cause of the difference is given by the dissociation of the solutes. It may be formulated as follows:

1. Aqueous solutions of all heavy metals, in which the cation alone contains the metal, produce the same distorting effect on the nuclei (fig. 1). Addition of ethyl alcohol, isopropyl alcohol, normal propyl alcohol, ether, formalin, phenol, ortho-, meta-, para-nitrophenol or parabromophenol in no way modifies or checks the effect.

2. Aqueous solutions in which the anion alone contains the metal give normal fixation of nuclei and the same holds true in case of solutions of salts in which one metal forms part of the anion, while another metal forms part of the cation. Addition of formalin makes no difference (fig. 2). Of the many fixing fluids containing potassium bichromate and therefore belonging to this type, Regaud's fluid gives in my experience the most satisfactory fixation. Neither the formulae

proposed by Zirkle, nor such experimented with by myself, equal it. Unfortunately Regaud's fluid has serious faults: it is unstable and begins to decompose within a few hours, its rate of penetration is so low that only very small pieces of tissue can be fixed and it has the tendency to harden tissues excessively. Notwithstanding such drawbacks it is used extensively for the study of mitochondria which it preserves well. But mitochondria may be even better preserved by salts in which the metal forms part of the cation if phenol is added to the solution. In such mixtures even cobalt gives good results, while copper, mercury and uranium are superior to potassium bichromate. Thallium, too, may be used to demonstrate mitochondria. Its salts give strongly alkaline solutions of pH 10 and even 10.8 and must be adjusted to pH 7 by the addition of hydrochloric acid. Even so the fixation of structures other than mitochondria is not satisfactory and the cells look empty.

Antimony salts have a peculiar effect on the staining properties of the cytoplasm of amphibian erythrocytes. The cytoplasm becomes glass-clear and neither eosin nor acid fuchsin have any power to stain it. This is unexpected in view of the fact that antimony trifluoride was used in the past as a mordant in the textile industry. Several other undesirable effects of antimony on fixation of cytoplasm and chromatin, as well as the instability of its salts make its use troublesome and inferior to several other elements.

The most satisfactory salts in my experience are the salts of copper. It is true that, alone, copper salts do not precipitate proteins as fully as do salts of mercury. But when used in combination with acids and phenols complete precipitation may be accomplished. The use of picric acid (trinitrophenol) and trichloroacetic acid for this purpose cannot be recommended although both are powerful precipitants of proteins. I have tried both in many combinations. Picric acid affects the cell structures in a manner which makes differential staining impossible unless one is interested only in chromosomes. Trichloroacetic acid forms a regular ingredient of the

so-called "Susa" fixing fluid proposed by Heidenhain. It leaves cells empty. I have tried it in numerous combinations with other ingredients and had to discard it because of its deleterious effect on cytoplasm. Ripart and Petit introduced cupric chloride and cupric acetate as ingredients of their fixing fluid. Cupric nitrate is an ingredient of my original paranitrophenol fixing fluid. All soluble salts of copper serve as powerful mordants for many stains and do not harden tissues to the extent to which chromium, mercury and uranium do.

But let us return to the problem of checking the condensation effect of chromatin by fluids in which the metal forms part of the cation. Addition of nitric acid does it to a certain degree, but not fully. Nitric acid, a strong oxidizer, forms part of my paranitro fixing fluid. In combination with paranitrophenol it helps complete precipitation of proteins, increases the speed of penetration and counteracts excessive hardening. Acetic acid does not give as complete precipitation, but if added to an aqueous solution of a metallic salt such as cupric nitrate or cobaltous nitrate, it prevents the condensation of chromatin and induces normal fixation of nuclei. But acetic acid destroys mitochondria or else makes them invisible. It also has a bad effect on erythrocytes and in some fixing fluids destroys them completely. However, neither acetic acid, nor its haloid derivatives destroy mitochondria if used in combination with phenol or its derivatives. The same is true of propionic acid. Instead, these acids introduce another deleterious effect, breaking up the cytoplasm of amphibian erythrocytes into globules or granules (fig. 3). After considerable experimentation I have found that this can be easily prevented by the simple addition to or substitution in place of acetic acid of formic acid. The latter is a strong reducer, prevents the lumping of chromatin in the nuclei and the breaking up of the cytoplasm (fig. 4).

The French edition of Lee's *Microtomists's Vade-Mecum* published by him in collaboration with Henneguy in 1896, contains on page 59 a statement not found in the American

editions. This statement refers to the use of bromine water in connection with Ripart and Petit's fixing fluid for fixation of cells rich in protoplasm. As a student in Moscow University I tried this method the same year that the book was published. At that time I worked on cockroaches and found difficulties in getting good fixation of their salivary glands. The use of Ripart and Petit's fluid with the recommended addition of bromine water proved to be a wonderful improvement. I still have in my possession a slide which I made at that time and I have never since been able to get as good fixation by any of the known fluids, my own sublimate and paranitro included. With this experience in my mind I decided to try out the introduction of a bromine ion in the new fixing fluids with which I was experimenting. This time I did not use bromine water as recommended by Ripart and Petit, but selected the bromophenols, bromacetic acid and cupric bromide because of the greater convenience in preparing solutions. Of the bromophenols only parabromophenol can be used. The combination of the three in an alcoholic solution gives excellent fixation, possesses great penetrating power and leaves tissues soft. The granulation of erythrocytes is prevented by the addition of formic acid. Resting and dividing nuclei are well fixed (fig. 5). Mitochondria show up clearly, especially if stained in iron-haematoxylin without any counterstain, thus leaving the rest of the cell colorless (fig. 6). In fact, this fluid gives better fixation of mitochondria than does Regaud's bichromate-formalin. Mitochondria in the mammalian liver are also preserved. What is still more interesting is the fact that bile canaliculi become easily visible, something very unusual in the case of fixation without previous injection of the ducts (fig. 7). On the whole I think I have finally solved the problem of making a fixing fluid which would leave the tissues soft and at the same time give good nuclear and cytoplasmic fixation.

The formulae of three new fixing fluids are given below. Of these no. 1 is a modification of my old no. 1 phenol-cupric fixing fluid with that difference that it is stable and may

be prepared in a single solution. It is free of the distorting effect on the fixation of nuclei. Formula no. 2 is a modification of my old no. 2 paranitrophenol-cupric. It is stable, can be prepared in a single solution and gives normal fixation of nuclei. Both fluids are rapid in their action and leave the tissues about as soft as the old fluids. Either ethyl alcohol or iso-propyl alcohol may be used. Normal propyl alcohol may also be used, but has no advantage and is more expensive. I have reduced the strength of the alcohol from 60% to 40% because experience has shown that the weaker solution is sufficient to prevent even insect eggs from floating and is less injurious to tissues rich in water. However, the strength may be increased or decreased to meet special requirements.

Formula no. 3, the bromophenol-cupric fixing fluid, gives better fixation and leaves tissues still softer, but is more expensive and less stable when prepared in a single solution. In about 10 days a colorless, oily, heavy fluid appears at the bottom, apparently belonging to a bromine lacrimator type of compound, highly irritating to the eyes. If the solvent is iso-propyl alcohol the decomposition of the fluid is somewhat slower, taking not less than 3 weeks at room temperature. It is better, therefore, to prepare the fluid in two stock solutions as given in the instructions and to mix them at the time of use. Silver impregnation by the Bodian method gives excellent results after fixation in the bromophenol-cupric fluid. If no. 1 or no. 2 is to be used for subsequent silver impregnation, a solution of pure formaldehyde, ca. 37% strong, should be added in a ratio of no. 1 (or no. 2) 4 parts, formaldehyde 1 part. Formaldehyde cannot be added to the bromophenol cupric fluid, as the mixture decomposes rapidly, but as just stated, the fluid permits excellent impregnation when used alone.

The relative proportion of the ingredients was worked out by trial. In some cases the amount of the 40% alcohol may be increased from two to five times. However, the penetration of the fluid is then less rapid and while that may be desirable in some cases, it should be avoided whenever possible. I use

such weak solutions for the fixation of complete specimens of invertebrates which are afterwards either to be cleared and left in oil for purposes of demonstration of their internal anatomy, or are to be used for dissection in weak alcohol. The fixation is so gentle that the tissues remain semitransparent. If a large earthworm is first cleared of all soil in its intestine by keeping it for days between wet sheets of muslin, then anesthetized and allowed to stretch by putting it into 5% alcohol with some ether at a temperature slowly increasing to 50°C. and fixed in a weak solution of any of the three fixing fluids, washed and transferred into 70% alcohol, it may remain in the alcohol for many weeks retaining its flexibility to such an extent that it may be wound up spirally without damage and regains its shape when released. Dehydrated and cleared in cedar oil or xylene, almost the entire anatomy of the worm becomes visible. In the case of insects fixed in one of the new fixing fluids, dissection in weak alcohol or water is greatly facilitated because the malpighian tubules, while remaining soft and semitransparent, acquire sufficient tensile strength not to be broken while being disentangled.

Tissues are best allowed to remain in the fixing fluid for several hours or over night. They may be washed in running water or in weak alcohol, increasing the strength gradually to avoid shrinkage in view of their softness after fixation. In my paper published in *Science* in 1933 I pointed out that tissues fixed in the paranitrophenol-cupric mixture increase about 16% in volume after they are transferred into 70% alcohol. No explanation of this phenomenon was offered. I depended upon the displacement of water before and after fixation. Recently Stowell applied a different method of measurement consisting in careful measurement of the area of one surface of a piece of kidney and calculating the change in volume. He came to the conclusion that tissues fixed in the paranitro mixture increase by about 30% in volume in 70% alcohol and then shrink in the process of embedding in paraffin to about 64% of their original volume. He further found

similar considerable swelling of tissues fixed in formalin. The final shrinkage amounts in the case of formalin fixed tissues to 60% of the original volume. Bouin's, Susa and Zenker produce no swelling, but cause a final shrinkage to from 47% to 63% of the original volume. I cannot agree with Stowell's conclusion that the quality of fixation has anything to do with swelling and shrinkage of tissues. These are strictly physical phenomena. Shrinkage after most fixing fluids is considerably greater than after paranitro. The new bromophenol-cupric fixing fluid causes slightly greater swelling than the old paranitro, yet fixation is much better. The fact is that good fixation depends upon proper combination of salts of heavy metals with acids and other ingredients, upon their rate of penetration and upon their chemical action on the cell constituents. Swelling and shrinkage are undesirable, but they are equally injurious in good as in bad fixation.

No. 1. Phenol-cupric fixing fluid, pH 1.5

40% ethyl (or iso-propyl) alcohol	100 cc.
Ether	5 cc.
Formic acid, spec. grav. 1.20, ca. 87%	5 cc.
Phenol	5 gm.
Cupric bromide (or nitrate)	3 gm.

No. 2. Paranitrophenol-cupric fixing fluid, pH 1.5

40% ethyl (or iso-propyl) alcohol	100 cc.
Ether	5 cc.
Formic acid, spec. grav. 1.20, ca. 87%	5 cc.
Paranitrophenol	5 gm.
Cupric bromide (or nitrate)	3 gm.

No. 3. Bromophenol-cupric fixing fluid, pH 1.35

Solution A.

40% iso-propyl (or ethyl) alcohol	100 cc.
Ether	5 cc.
Formic acid, spec. grav. 1.20, ca. 87%	10 cc.
Cupric bromide	6 gm.

Solution B.

40% iso-propyl (or ethyl) alcohol	100 cc.
Ether	5 cc.
Bromacetic acid	10 gm.
Parabromophenol	10 gm.

For use mix: Solution A — 1 part by volume, Solution B — 1 part. If desired, add 40% iso-propyl (or ethyl) alcohol from 1 to 5 parts by volume.

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PLATE

PLATE 1

EXPLANATION OF FIGURES

Figures 1 to 6 are microphotographs of sections through tissues of the common newt, *Triturus viridescens* Raf., made at a linear magnification of 700. Figure 7 is a microphotograph of a section through the liver of cat at a linear magnification of 1300.

1 Effect of metal cations on nuclei. Piece of skin fixed in a solution of cupric nitrate in 40% alcohol with the addition of some formalin. Stained in haematoxylin-eosin. Notice the condensation of chromatin at one end of the nuclei. The same effect is produced by similar salts of cobalt, mercury, uranium etc.

2 Effect of metal anions on nuclei. Piece of skin fixed in Regaud's potassium bichromate-formalin fluid and stained in haematoxylin-eosin. Notice that the nuclei appear normal.

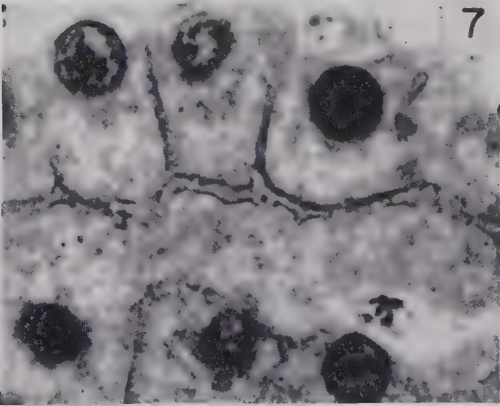
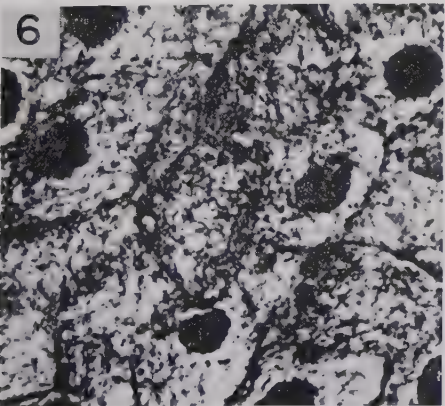
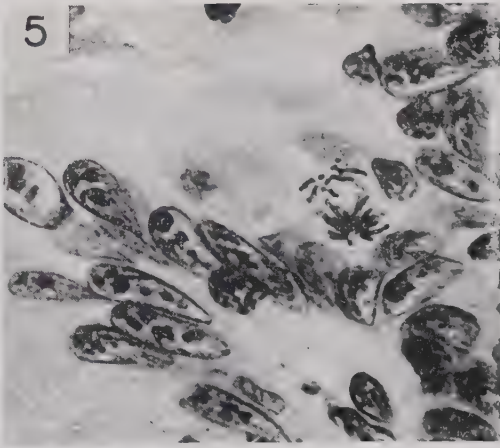
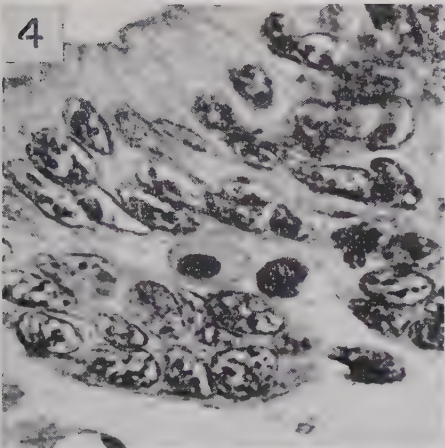
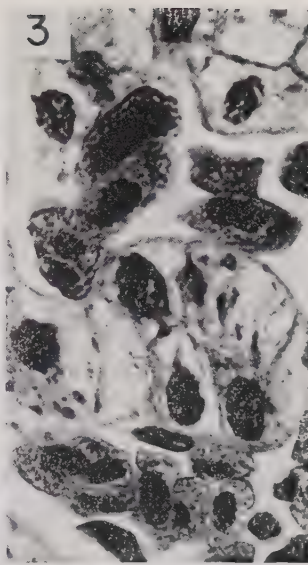
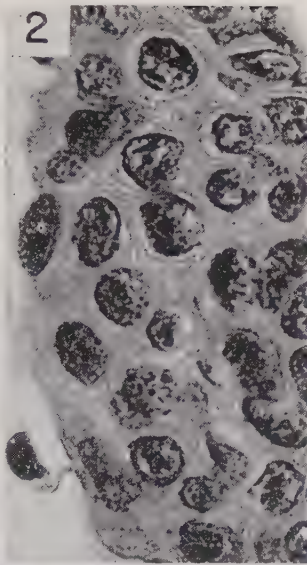
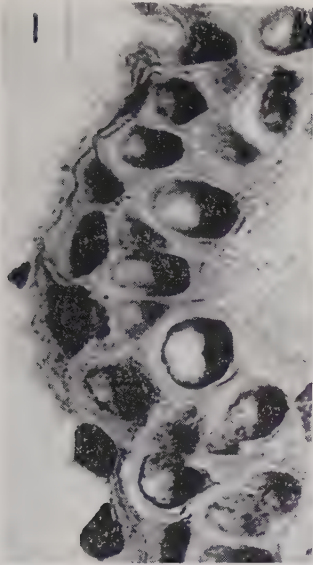
3 Effect of acetic acid and its derivatives on the cytoplasm of erythrocytes in presence of phenols. Piece of liver fixed in an alcoholic solution of cupric bromide, bromophenol and bromacetic acid. Stained in haematoxylin-eosin. Notice the granulation of the cytoplasm.

4 Correcting effect of formic acid on fixation when added to the same fixing fluid used in figure 3. Skin, stained in haematoxylin-eosin. Notice normal appearance of nuclei and cytoplasm.

5 Intestine fixed in no. 3 bromophenol-cupric fixing fluid and stained in haematoxylin-eosin.

6 Piece of liver fixed in no. 3 bromophenol-cupric fixing fluid and stained in iron-haematoxylin without counterstain. Nothing but nuclei and mitochondria appear stained.

7 Piece of cat's liver fixed in no. 3 bromophenol-cupric fixing fluid and stained in iron-haematoxylin. Notice the bile canaliculi.



OVARIAN CYSTS IN IMMATURE FEMALE CATS FOLLOWING PREGNANT MARE SERUM HORMONE ADMINISTRATION

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ONE PLATE (ELEVEN FIGURES)

The formation of ovarian cysts following administration of gonadotropic hormone has been observed in many laboratory animals (Engle, '39; Hisaw, Hertz and Fevold, '36; Hisaw, Fevold and Leonard, '31). No report concerning the production of such cysts in the immature cat by similar treatment has come to our attention. Previously, we had observed that most immature female cats injected with menopause urine or male urine extracts, responded by essentially normal follicular development (Starkey and Leatham, '39, '40). In a recent study of a series of thirty-six immature cats treated with pregnant mare serum hormone, many cases of excessive ovarian stimulation with cystic formation were noted. Examples of the types of cysts observed and the corresponding uterine conditions are herein described.

The three immature cats to be discussed were 16 or 17 weeks old at the time of treatment. Each animal received a single subcutaneous injection of 400 International Units (200 Cole-Saunders Rat Units) of pregnant mare serum hormone (Gonadin¹).

¹ The pregnant mare serum, Gonadin, used in this study was supplied through the kindness of Dr. Donald H. Wonder of the Cutter Laboratories, Berkeley, California.

Cat no. 100. Laparotomy was performed 11 days after hormone administration. Both ovaries were observed to be greatly stimulated, many clear, mulberry-like cysts being present. The left ovary and a portion of the uterus were removed. This ovary weighed 1620 mg. (wet weight) as compared to 45 mg. for a nontreated control immature ovary. Sections of this ovary showed tremendous cystic distension with a compression of the stroma (fig. 1). An occasional discus proligerus and a few primordial follicles were seen. The numerous cysts were filled with secretion and were lined with granulosa cells, many of which were in various stages of degeneration (fig. 5). The endometrium was in a proliferative state with edema in the submucosa and only slight glandular stimulation. Perivascular edema was marked (fig. 9). On the twenty-third day, the right ovary and uterine horn were removed. This ovary weighed 1210 mg. and contained four large, fluid-filled cysts (fig. 2). On section, these cysts were seen to be lined with luteal cells, giving these structures the appearance of corpus luteum cysts (fig. 6). The corresponding uterus contained a well-developed progestional endometrium with tortuous glands extending into and replacing the submucosa (fig. 10). Perivascular edema was absent.

Cat no. 109. Laparotomy was performed on the eleventh day after hormone administration. Both ovaries were grossly observed to be enlarged and cystic as were the ovaries of cat no. 100 at this time interval. On the twenty-second day, the right ovary was removed and found to weigh 387 mg. Grossly it appeared to have regressed from the excessive cystic condition previously observed. The left ovary appeared similar. On section, the right ovary contained a large follicular cyst (fig. 3) which was lined by a single layer of cuboidal epithelium (fig. 7). In the surrounding stroma were small atretic follicles but no graafian follicles. The uterine endometrium was proliferative with an absence of glandular structures. There was no submucosal edema.

Cat no. 111. At laparotomy on the fifteenth day after treatment, the right ovary was noted to have the gross appearance of regression from the cystic type of stimulation which was initially observed in the other cats. The left ovary had four small, clear cysts at the anterior pole and one large, multilocular cyst in the central portion of the ovary. On the twenty-third day, the right ovary appeared completely regressed. The left ovary still contained cysts which had been observed on the fifteenth day. On the twentieth day, the left ovary was removed and weighed 164 mg. On section, it was found to have regressed and contained one cyst and many areas of atresia (fig. 4). This cyst was filled with an eosinophilic secretion in which there was much cellular debris (fig. 8). The wall was made up of a thickened layer of connective tissue and areas of hyalinization. The uterus showed a moderately proliferative endometrium with a slightly thickened wall and congestion of the submucosal capillaries (fig. 11).

DISCUSSION

The ovaries of all immature female cats receiving a large single injection of pregnant mare serum hormone (PMS) responded initially by tremendous cystic follicular development. These cysts may rapidly regress within a few days, remain as simple retention cysts or form luteal cysts. These latter are of particular interest since Allen ('42) has recently stated that "corpus luteum cysts occur spontaneously in some animals, but have not been produced experimentally."

The persistence of cysts, as observed by us in the cat, may be the result of the prolonged action of the pregnant mare serum hormone. The prolonged effectiveness of this hormone has been attributed to slow absorption, to slow destruction in the body and to the fact that there is little if any excretion of this gonadotropic material in the urine (Hamburger and Pedersen-Bjergaard, '38; Evans, Simpson and Austin, '33; Catchpole, Cole and Pearson, '35).

SUMMARY

Examples of the response of ovaries and uteri of immature female cats to single large injections of pregnant mare serum hormone have been described. The initial effect of such treatment is the production of large multilocular cysts containing clear fluid and lined with granulosa cells. The production of a proliferative state of the uterine endometrium indicates that such cysts probably secrete estrin.

Similar cysts have been noted to regress or to persist as simple retention cysts, which are lined by cuboidal epithelium, or to form corpus luteum cysts which are lined by luteal cells. That these luteal cells are secretory is shown by the production of a progestational endometrium. At 30 days after hormone administration, cysts in various stages of atresia have been noted.

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PLATE 1

EXPLANATION OF FIGURES

1 Cat no. 100. Ovary removed 11 days after pregnant mare serum hormone injection. Note many cysts and compression of stroma. $\times 8$.

2 Cat no. 100. Ovary removed 23 days after treatment. Note large corpus luteum cysts. $\times 8$.

3 Cat no. 109. Ovary removed 22 days after treatment. Note large retention cyst and numerous cystic follicles. $\times 8$.

4 Cat no. 111. Ovary removed 30 days after treatment. Note the atresia of the cyst and areas of hyalinization in the stroma. $\times 8$.

5 Cyst wall from ovary shown in figure 1. Note the granulosa cell lining of the cyst cavity. $\times 190$.

6 Cyst wall from ovary shown in figure 2. Note the lining of luteal cells. $\times 210$.

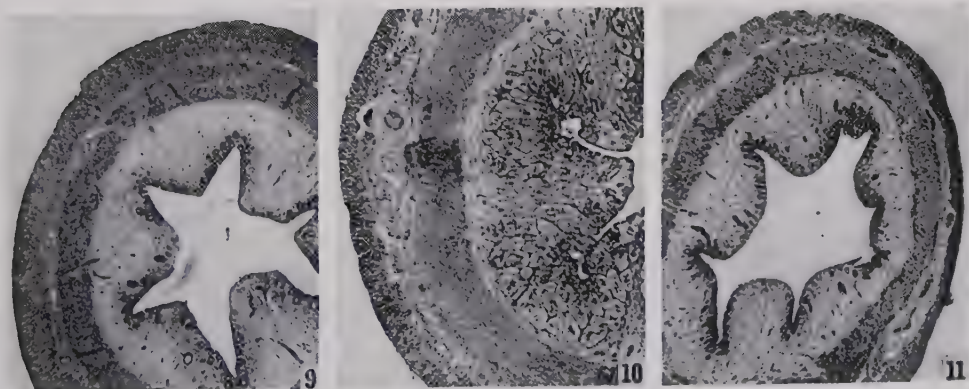
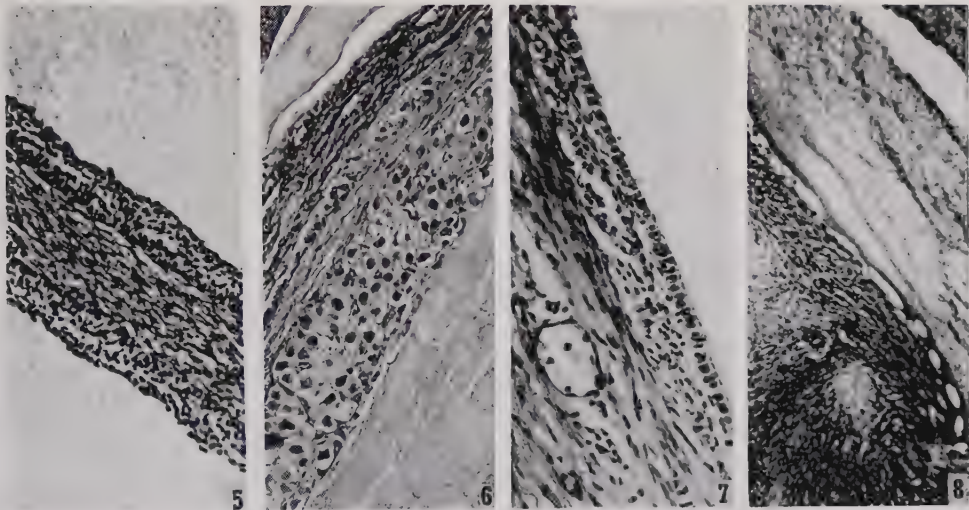
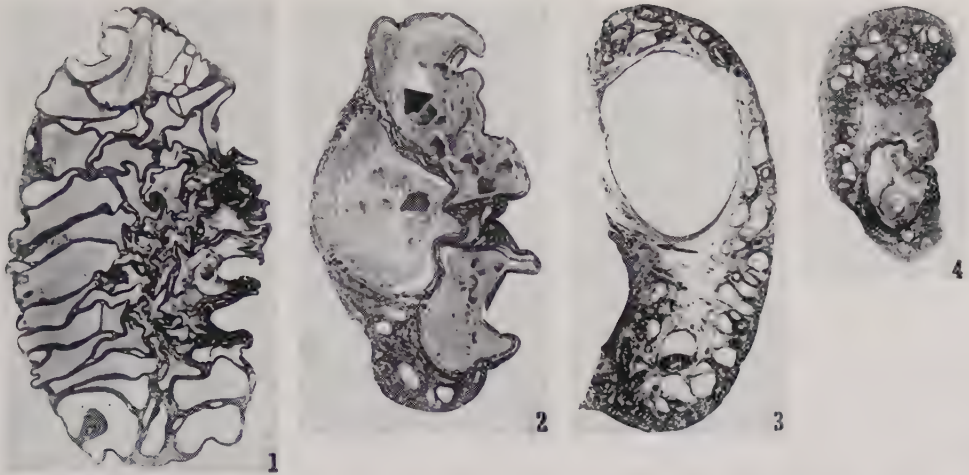
7 Cyst wall from ovary shown in figure 3. Note the single layer of cuboidal cells lining the cyst cavity. $\times 210$.

8 Cyst wall from ovary shown in figure 4. Note the extensive fibrosis and hyalinization of the wall. $\times 220$.

9 Uterus corresponding to ovary shown in figure 1. Note the proliferative endometrium, edema in the submucosa and slight glandular proliferation. $\times 60$.

10 Uterus corresponding to ovary shown in figure 2. Note the progestational endometrium with well developed glands extending into the submucosa. $\times 60$.

11 Uterus corresponding to ovary shown in figure 4. Note the moderately proliferative endometrium and lack of glandular stimulation. $\times 60$.



A STUDY OF THE CYTOGENESIS OF CORTICO-ADRENAL CELLS IN THE CAT

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TWENTY-THREE FIGURES

INTRODUCTION

Recent studies on a series of adrenal glands of primates, carnivores, rodents and amphibians by Zwemer, Wotton and Norkus ('38) led them to postulate that the cortical gland cells may be derived from certain indifferent cells of the capsule; and that the various cell types found in the cortex are continuous stages in the life history of the same cell. Baker and Baillif ('39) studying the regeneration of enucleated adrenal glands arrived at a similar conclusion; that the cortical cells and their supporting tissues arose from cells in the capsule of the gland. Using colchicine to fix mitoses in normal glands, Tizlowitz (personal communication) came to the same conclusion. Our present observations on the morphogenesis of the cytological components in the capsular and subcapsular cells support the evidence advanced above for the origin and differentiation of new cortical cells from the capsule of the adrenal gland.

TECHNIQUE

Adrenals from young, rapidly growing animals show differentiation of gland cells from the capsular cells, better than

the more static adult glands. Therefore, whole glands from 10-day and 8-week-old kittens being sacrificed by their owners, were rapidly removed at necropsy. All the animals appeared normal while alive and revealed no gross pathological changes at autopsy. Because the glands were sufficiently small it was considered advisable not to maltreat them by division or other manipulation before fixation. Champy's fluid (Lee, '37) was employed as a fixing agent for all the adrenals used in this study. To demonstrate the Golgi reducing substance¹ (figs. 1 to 6, and 19) some of the glands were osmicated at 35°C. for 9 days; while others were carried through methods proposed by Severinghaus ('32) preparatory to staining for mitochondria (figs. 7 to 13). The most useful preparations were obtained by superimposing the Severinghaus stain upon osmicated tissues. In this way both the Golgi and mitochondria could be observed simultaneously in the same section.

OBSERVATIONS

The Golgi reducing substance. From the periphery of the capsule inward, long, thin, connective tissue-like cells are seen in various intermediate stages of differentiation into the thick polyhedral cells of the subcapsular (glomerular) region. When capsule cells in sequence are observed to become shorter and filled with many large, refractile granules, this compact mass of Golgi is seen to thin out and become more diffuse and irregular in contour (figs. 1, 19 to 23). In the subcapsular cells these granules lie within the lamellar structure of the reducing material (figs. 5, 6, 15 to 18, and 21).

The Golgi is always found to lie in a position between the nucleus and the capillary margin of the cell. And according to recent criteria for classification by Pollister ('39), the "Golgi apparatus," in the undifferentiated cortical cell of

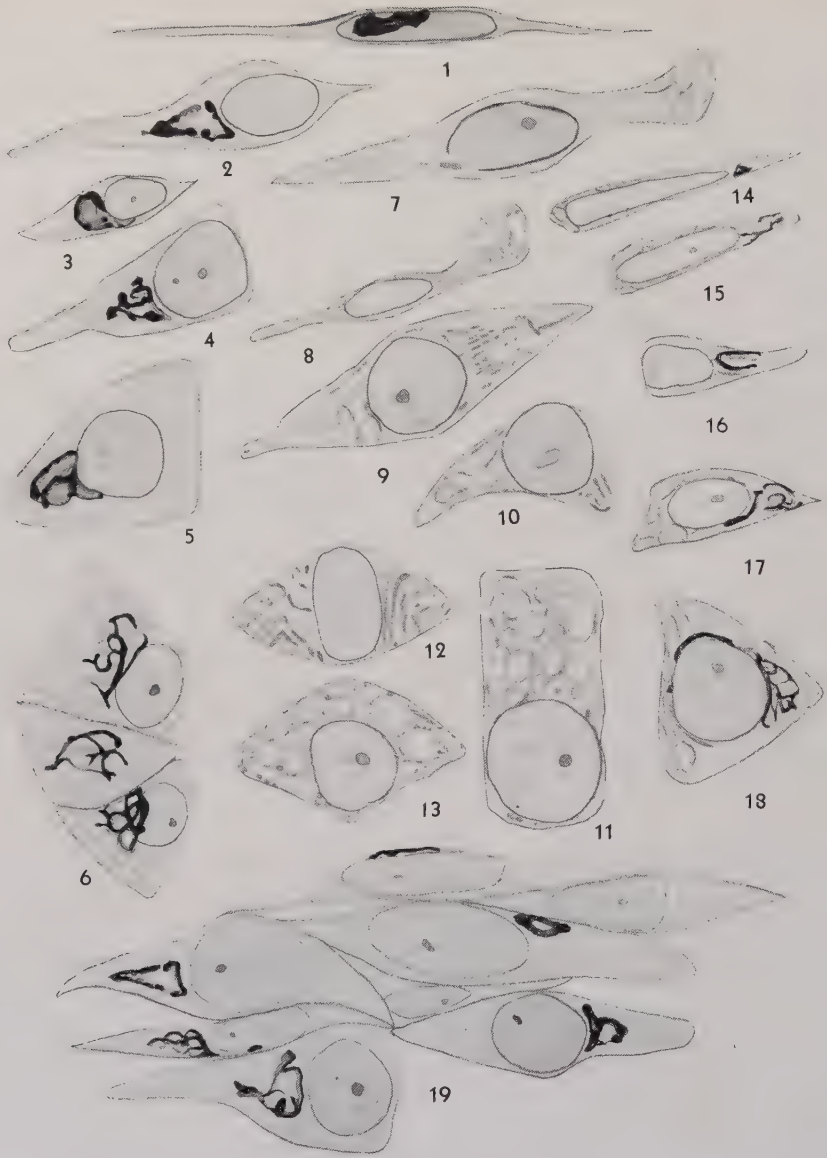
¹ This material subsequently will be referred to as Golgi as we feel the words Golgi apparatus or Golgi body, connote a mechanistic rather than a physico-chemical concept of the living cell.

the cat adrenal, partakes of the characteristics of the leucocyte type. It consists of a single mass, lamellar in structure, lying in close contact with the nucleus. As in the leucocyte, the centriole is situated within the ring of the Golgi substance.

Origin of new cell columns. The origin of new, gland-cellular foci in the capsule of the adrenal gland may be predicted by the distribution of the Golgi. It can be seen, in a given group of cells in the capsule, that the orientation of the Golgi material follows approximately the periphery of a semi-circle (figs. 19 and 21). Such a cellular configuration gives rise to a column of cells which maintains its continuity throughout the cortex of the gland. A sector of a cross section of a column in the outer fascicular region is shown in figure 6. Detailed discussion of these cell columns is given by Zwemer, Wotton and Norkus ('38; their fig. 2). In the corticoadrenal cell the Golgi substance is situated on the capillary side of the nucleus so that it always lies peripherally in the cell columns. This position of the Golgi in the cell of the adrenal cortex is opposite to that found in a typical exocrine gland cell as regards the capillary surface, but in both types the Golgi is closest to the secreting surface.

The mitochondria. From the outer capsule cells progressively inward the mitochondria become more numerous with differentiation. Only one or two mitochondria are visible in the long capsular cells at the time their Golgi material is a closely compact mass (figs. 7 and 14). A large increase in the number of mitochondria is found concomitantly with the expansion of the Golgi substance (figs. 11 and 18).

Refractile granules. The refractile granules first become visible in the cytoplasm among the mitochondria (fig. 7) and when seen at later stages in the region of the Golgi, they appear to have increased in size. When studied in the fascicular region the corticoadrenal cell usually is seen to be packed densely with lipoid droplets, the Golgi is very diffuse, and numerous mitochondria fill the interspaces in the cytoplasm.



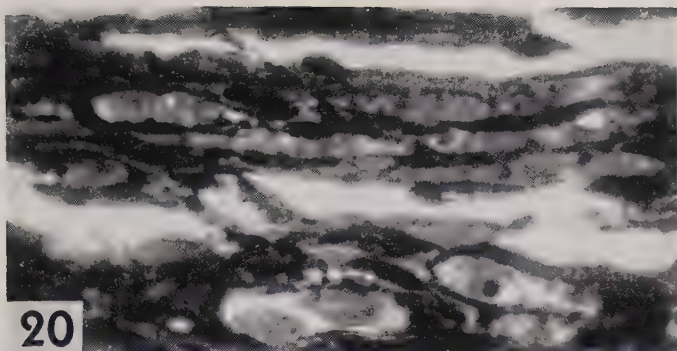
Figs.1 to 19 Drawings of Golgi reducing substance and mitochondria in corticoadrenal gland cells. All figures were outlined with the camera lucida; the details were filled in free-hand.

Figures 1 to 6 and 19 were drawn from Kolatchew preparations for Golgi material.

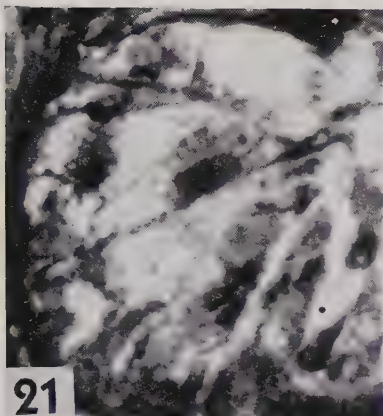
Figures 7 to 13 are from material stained for mitochondria by the Severinghaus method.

Figures 14 to 18 showing both Golgi and mitochondria were drawn from tissue which had been osmicated and subsequently treated according to Severinghaus.

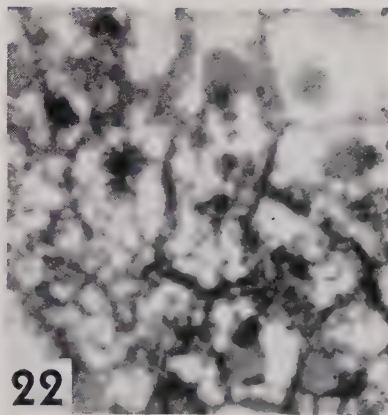
All figures approximately $\times 2000$.



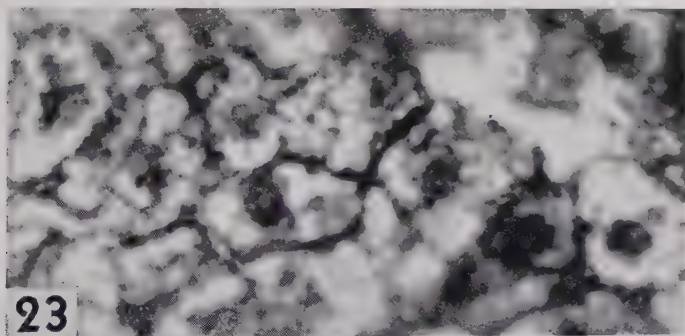
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Figs. 20 to 23 Photomicrographs of Kolatchew preparations of the adrenal cortex from a series different from those drawn, with no collusion between artist and photographer. $\times 1500$.

Figure 20 Capsule.

Figure 21 Subcapsular cells showing transition from compact to diffuse type Golgi.

Figure 22 Adrenal cortex cells with moderate dispersion of the reducing substance.

Figure 23 Adrenal cortex cells with extensive dispersion of the reducing substance.

DISCUSSION

The derivation of adrenal cortex gland cells from the capsule was first reported by Zwemer ('34) based on morphological grounds. Confirmations by studies of pathologic adrenal material (Zwemer, '36) and of glands after experimental procedures (Zwemer, Wotton and Norkus, '38) was strengthened by colchicine studied on both normal (Tizlowitz, personal communication) and transplanted adrenal capsules (Baker and Baillif, '39). Because capsule cells take up trypan blue and other vital dyes, their differentiation into gland cells can be followed chronologically (Salmon and Zwemer, '41).

In the present studies we find that cytogenesis is closely synchronized with histological development, and that alterations in cell components, such as the Golgi reducing substance and the mitochondria are indicative of approaching cellular differentiation and secretory activity.

Hirsch ('39), working on the secretion cycle in living cells of the mouse pancreas, advanced evidence that mitochondria are responsible for the origin of secretion products in the form of small granules. After their primary appearance among the mitochondria, he observed that the granules migrated into the region of the Golgi substance and grew in size. Duthie ('33), using fixed material from the pancreas, has in many ways confirmed the observations of Hirsch.

In the adrenal cortex also, the mitochondria are the first to appear active. As their number increases more refractile granules become visible in that part of the cell where mitochondria are present. This granular material seems to be accumulated first near the mitochondria and later is found also in the region of the Golgi where the individual droplet size is considerably augmented (figs. 14 to 17).

We agree with Kirkman and Severinghaus ('38), that the Golgi reducing substance is part of a complex functional system in the cell; although we differ from them in not stressing an organelle nature for this material. The fact that

it can be moved from one part of a cell to another by ultracentrifugation (Dornfeld, '36, '37) indicates chiefly its uniformity of composition and specific gravity.

Bourne ('34), in his studies on the corticoadrenal Golgi material, found two distinct distribution types; one compact, and the other diffuse which he believed to be derived from the compact form. He did not, however, emphasize the transitional forms which connect up this sequence with cellular differentiation.

The position and topography of the mitochondria and the Golgi in the cell are characteristically based on function according to Pollister ('32). We believe that their orientation in the cells of the adrenal capsule may be used to identify new foci of gland cell formation, and that their physicochemical reactions within any given gland cell are associated with the state of its secretory activity. Furthermore, the distribution of mitochondria and Golgi substance in corticoadrenal cells of the cat indicates that secretion products are elaborated through only one metabolic cycle of differentiation; accretion of material by the cell, its elaboration into hormone, a secretion into the capillaries and finally, degeneration.

SUMMARY

Studies on the cytological components of capsular and subcapsular cells of the adrenal cortex of cats have been made. These studies support the view that corticoadrenal gland cells may be derived from certain indifferent cells found in the capsule of the gland, and that the various morphological types found are different stages in the life history of the same cell.

Indication of new foci of gland cell formation in the capsule of the adrenal cortex is given by the position and topography of certain cytoplasmic elements such as the Golgi material and the mitochondria. The physical distribution of these cell components is also associated with the metabolic activity of differentiation and secretion.

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STUDIES ON THE PRODUCTION OF SECONDARY DECIDUOMATA DURING LACTATION IN THE RAT

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ONE PLATE (THREE FIGURES)

The effect of progesterone in the rat has usually been studied by means of the decíduoma reaction. It is well established that the endometrium is sensitized by progesterone so that decíduomata can be produced experimentally, and conversely, that whenever decíduomata appear as a result of trauma it is direct evidence that the corpora lutea are functional. However, failure to obtain decíduomata following trauma is not necessarily evidence that the corpora lutea are not functional or that progesterone is not acting. In the sterile horn of a unilateral pregnancy, for example, decíduomata can be produced by trauma only over a short period of time, from the fourth to the seventh days (Allen, '31), yet the corpora lutea are functional throughout the major portion of gestation. The rationale for the lack of sensitization at later periods of gestation is not well established but it seems probable that in the latter part of gestation the amounts of estrogen and progestogen being produced by the ovaries, and possibly by the placenta, are not satisfactory for the continuation of sensitization of the endometrium, yet it is obvious that the amounts produced are ideal for the continuation of pregnancy. It also seems probable that the lack of continued sensitization may be produced by excess

estrogen, since it is well known that the sensitizing effect of progesterone can be completely prevented by adequate amounts of estrogen.

A somewhat different balance of the hormones seems to be present during lactation. Ovulation occurs soon after parturition in the rat and the corpora lutea which result are functional. If nursing is permitted these corpora remain functional for approximately 3 weeks as shown by persisting sensitization of the endometrium as long as the seventeenth day (Long and Evans, '22; Lyon and Allen, '38). In both pregnancy and lactation the corpora lutea remain functional, but during pregnancy the uterus is sensitized only for a period of about 3 or 4 days, whereas during lactation the sensitization lasts much longer. There are other differences. During lactation the endometrium does not show late pregnancy changes such as are seen in the sterile horn of a unilateral pregnancy, and the vagina shows very slight mucification in contrast to the marked mucification present in pregnancy. Deciduomata produced during lactation are also usually smaller than those produced during pregnancy (Lyon, '39). It seems unlikely that the small size of deciduomata and the persistent sensitization of the endometrium during lactation are due to a high estrogen level since the vagina should then show excellent mucification. It seems more likely that the persistent sensitization during lactation is due to a low level of estrogen.

There is one other type of evidence which has to be considered. Deciduomata can be produced by traumatization at any time from the third to the seventeenth days of lactation, yet if deciduomata are produced by traumatization of one horn on the fifth day, traumatization of the other horn on the tenth day does not usually result in the formation of well developed deciduomata. This has been cited as indicating that failure of the second stimulation is due to too much estrogen, the source being the decidua regardless of whether the decidua be produced experimentally or because of pregnancy (Selye, '35). If this is so, it would indicate that the

decidua rather than the trophoblast is the source of placental estrogen in pregnancy. If the two are similar situations, namely, pregnancy, and lactation with deciduomata, the vagina should be highly mucified and the endometrium should show late pregnancy changes several days after the production of deciduomata during lactation. We have not found this to be the case and hence we have been disinclined to accept without reservation the above explanation for the inability to produce secondary deciduomata.

The more likely explanation, on the basis of anatomical findings, is that the ovaries during lactation produce a small amount of estrogen, and that the amount produced is inadequate to support, in co-operation with progesterone, two groups of deciduomata. If such be the correct explanation, the administration of small amounts of estrogen following the production of primary deciduomata should maintain sensitization of the endometrium so that a second stimulus would result in secondary deciduomata. The experiments recorded in this paper support this explanation.

METHODS

Rats of the Long-Evans strain were used after they had delivered normal litters. On the fourth or fifth day following delivery the endometrium of one horn was stimulated by passing a needle and silk thread through the uterus, a portion of the thread being left to identify the site of trauma. Five days later the animals were subjected to a second operation and the other horn was traumatized in a similar manner. The animals were permitted to nurse the litter throughout the period of observation. Various amounts of estrone (dissolved in olive oil) were given, the dose ranging from 1 μ g. to 10 μ g. daily. After numerous trials it was found that the administration of estrone for a period of about 3 days, the first dose being given before the second operation, and one dose the day following, permitted the formation of secondary deciduomata. When doses of the magnitude of 4 μ g. were continued for 3 days after the second operation, deciduoma

production was inhibited. The animals were sacrificed either 4 or 5 days after the second operation.

RESULTS

In all cases under consideration primary deciduomata were formed in one horn as a result of trauma on the fourth or fifth post partum day. Secondary deciduomata, however, were found only in those animals receiving 12 to 13 μ g. given over a period of 3 or 4 days usually beginning 1 day before the second operation. With this dose deciduomata were formed in twenty out of twenty-six animals (table 1).

TABLE 1

Effect of various amounts of estrogen on the production of secondary deciduomata in the lactating rat.

NO. OF RATS	MICROGRAMS OF ESTRONE GIVEN ON POST PARTUM DAYS							TOTAL ESTRONE MICROGRAMS	NUMBER SECONDARY DECIDUO- MATA	PER CENT HAVING SECONDARY DECIDUOMATA
	6	7	8	9	10	11	12			
5	0	0	0	4	4	2	0	10	0	0
3	0	0	1	2	3	4	0	10	0	0
16	0	0	0	4	4	4	0	12	13	81
5	0	0	4	4	4	0	0	12	3	60
5	0	1	4	4	4	0	0	13	4	80
6	0	2	2	2	4	4	0	14	0	0

Primary deciduomata produced in all cases by traumatization on the fifth post partum day.

Secondary trauma produced on the tenth post partum day.

DISCUSSION

The results obtained indicate that the failure to obtain deciduomata from a second stimulation of the endometrium on the tenth day is apparently due to a lack of estrogen, since the administration of estrogen under these conditions results in sensitization of the endometrium. The results indicate also that primary deciduomata not only do not produce estrogen, and thereby prevent the formation of secondary deciduomata because of excess estrogen, but rather that they probably actually utilize estrogen and thereby produce

a relative insufficiency of estrogen. This latter deduction is logical because, if primary deciduomata are not already present, stimulation on the tenth day results in the formation of deciduomata. The fate of estrogens in the body after injection, and especially their fate in the reproductive organs is still obscure. It would not be illogical, however, to suppose that the uterus with a growing tumor in it (deciduoma) might utilize more estrogen than a uterus which was not actively growing.

The mechanism by which estrone maintains sensitization, after primary deciduomata have been produced, requires further study. It is just as logical to suppose that primary deciduomata utilize progesterone as it is to suppose that they utilize estrogen. If such were the case failure to obtain secondary deciduomata could be due to progesterone deficiency. If that be the mechanism, one has to assume that the injection of estrogen maintains the activity of the corpora lutea at a higher level. This also is not unlikely since it is well known that estrogen prolongs the effective life of the corpora lutea of both pseudopregnancy and pregnancy in the rabbit (Allen and Heckel, '36; Heckel and Allen, '39). In fact there is some experimental evidence that there may be a relative progesterone deficiency during lactation. Weichert ('40) has found that the administration of progesterone to the lactating, pregnant rat tends to prevent the delay of implantation.

These results, although of different type, are in accord with recent observations of Krehbiel ('41) and Weichert ('42). They have shown that the delay of implantation during lactation can be prevented by the administration of estrogens. Krehbiel has shown also that during lactation the embryos implant at either the normal time or at intervals of 4 to 5 days thereafter, as if the ovary were exhibiting a periodicity similar to that of the normal cycles even though there are functional corpora lutea in the ovaries. A similar periodic growth of follicles is known to occur during pregnancy also (Swezy and Evans, '30). It appears, therefore, that failure

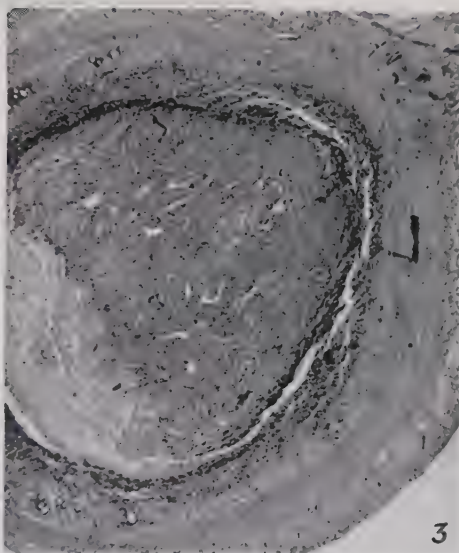
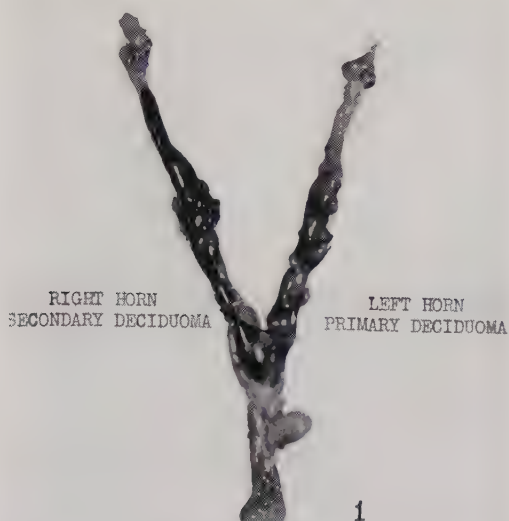
of implantation during lactation and failure to obtain secondary deciduomata as in the experiments recorded, are both due to a relative estrogen deficiency. In both instances the administration of estrogen, acting in conjunction with progesterone, renders the endometrium sensitive.

SUMMARY

Secondary deciduomata were obtained in lactating rats following trauma on the tenth post partum day, primary deciduomata already being present as a result of trauma on the fourth or fifth post partum day. These secondary growths were secured by injecting 12 to 13 μ g. of estrone over a period of 3 or 4 days, beginning usually on the eighth or ninth day post partum.

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- 1 Primary and secondary deciduomata in opposite horns of lactating rat.
- 2 Photomicrograph $\times 120$ of secondary deciduoma, from right horn shown in figure 1.
- 3 Photomicrograph $\times 120$ of primary deciduoma, from left horn shown in figure 2.

THE INTRACELLULAR LIPIN, MUCOID, AND GLYCOGEN OF THE VAGINAL EPITHELIUM OF THE GUINEA PIG¹

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TWO PLATES (TEN FIGURES)

INTRODUCTION

Our present knowledge concerning the cyclic changes in the vaginal epithelium has been derived, to a large extent, from observations on the sexual cycle of rodents. As early as 1887, Lataste, recognized that the vaginal epithelium of the rat periodically underwent "cornification" and subsequent desquamation at estrus. The following year Morau described the superficial "mucus" cells which characterize the vaginal epithelium, but Barrington ('15) appears to have been the first to demonstrate that these cells (gravid rat) stain intensively with mucicarmin. His observations were confirmed by Tsu ('24) for the rabbit and by Cole ('30) for the cow. This evidence is suggestive although no saliva-digested controls were used to differentiate glycogen from mucus in these studies. Long and Evans ('22) have described a layer of cells ("stratum granulosum") in the vaginal epithelium of the rat which contains hematoxylin-staining granules during the early part of estrus. Since there are no microchemical tests or specific stains for keratin it has been assumed that the "cornified" cells of the vagina undergo a process comparable to similar cells in the epidermis, and that these granules represent keratohyaline. The existence of glycogen

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in the vaginal mucosa of rodents does not seem to have been studied, although it has long been known that primate vaginal epithelium contains glycogen. Likewise, no report has been made on the lipin constituents of vaginal epithelium other than the observation of Hartman ('23) who found that many of the "cornified" cells desquamated from the opossum's vagina contain lipin droplets.

It is interesting to note that as early as 1914 Retterer and Lelievre concluded that the cells of the basal layer of vaginal epithelium had several potencies for differentiation. They also thought that some local or general stimulus (unknown) acted on the basal layer and modified the cytoplasm so that the cells were transformed into either a "mucous" or "cornified" type. Abundant evidence has been collected on this topic since Allen and Doisy ('23) showed that the active proliferation of the vaginal epithelium is due to estrin (cf. Allen et al., '39). Courier ('24) seems to have been the first to demonstrate that estrogen may produce a mucification of the vaginal epithelium in the guinea pig identical with that seen during proestrus or pregnancy. Meyer and W. Allen ('33) confirmed Courier and found that mucification is produced by estrin doses of about one-quarter to one-half of those required for the typical "cornification" reaction of normal estrus. Recently Markee and Berg ('42) have used mucification for assaying minute amounts of estrin and were able to make the first accurate determinations of the cyclic fluctuations of estrin in the blood of women.

The ovarian conditions with reference to the various stages of the guinea pig's cycle have been described in detail by Meyers et al. ('36). This study suggests that the rate of cell proliferation and differentiation in the vaginal epithelium parallels the rate of follicular growth in the ovary. That is, vaginal changes progress slowly during the first 12 days after estrus and then undergo a rapid acceleration during the 3 or 4 days before ovulation.

Stockard and Papanicolaou ('17) introduced the vaginal smear technique as an accurate method for determining the

duration of the guinea pig's cycle and correlating cytological changes in the vaginal fluid with concomitant changes in the ovary, uterus, and vagina. However, they failed to recognize a stage early in estrus when superficial mucous cells appear. Their stage I was characterized by the presence of cornified cells whose nuclei did not stain with hematoxylin. After the appearance of Long's abstract ('19) on the estrous cycle of the rat Stockard and Papanicolaou ('19) proposed to divide their stage I into an early phase characterized by nucleated and non-nucleated cells and a late phase in which only the latter cells occur. Selle ('22) has described a proestrous stage in the guinea pig characterized by typical nucleated superficial cells and relatively few leucocytes. His accurate study has recently been confirmed by Young ('37). It is evident from Papanicolaou's discussion in 1933 that he has failed to appreciate the fact that the superficial mucous cell is entirely different from all other vaginal cells which are simply the various phases in the metamorphosis of stratified squamous epithelium.

Recently the vaginal smear technique has been criticized by Young ('37) as not being as satisfactory in determining the time of estrus in the guinea pig as the behavioral response. He and his colleagues have concluded that ovulation is more closely related in time with the end of the period of receptivity than with any one of the stages they recognize in the vaginal smear. Meyers et al. ('36) report that ovulation usually occurs shortly before the end of estrus (sexual receptivity) when the latter is longer than 10 hours and shortly after its end when estrus lasts 7 hours or less. However, the only data they have published on this score (Young et al., '38, table 4), comes largely from cases of intermittent or "split" estrus. In a group of eleven animals killed 4.5 hours or less after the end of the second estrous phase only two had ruptured follicles. They do not seem to have tried to determine how early during estrus ovulation may take place. On the other hand, it appears that in a group of fifty-four animals over 50% had one or more typical vaginal cycles during which

the copulatory response could not be elicited. In this group 750 cycles are involved in which approximately 9% (66 cycles) had a negative mating response (Young et al., '39, p. 55). Furthermore, a group of thirty-three animals is described by Young ('37) which were killed when cornified cells were frequent in the smear; 89% of these had ovulated. When this evidence is taken into consideration it seems doubtful if the time of ovulation can be diagnosed more readily from mating behavior than from the vaginal smear, especially if the time of day is taken into account. Both of these methods, at the best, merely approximate the time at which ovulation may have occurred.

The object of the present study has been to determine the cyclic fluctuations of glycogen, lipin, and mucoid in vaginal epithelium and by the use of specific methods to provide data for the differentiation of vaginal cell types on a basis of intracellular components rather than on the vagaries of aniline dye staining. The guinea pig has been selected as especially favorable for this purpose.

MATERIAL AND METHODS

Fifty virgin guinea pigs, 6 to 10 months of age, were studied. They were kept in a warm (60–70°F.), light, well-ventilated room, and fed a constant mixed diet.

Selle's ('22) smear technique and classification were used. Vaginal examinations were made daily until the first estrous period was established. Only those animals that maintained a cycle within a range of 14 to 17 days for 2 to 3 consecutive cycles were utilized.

The vagina was removed under nembutal anesthesia, cut into thin (2–3 mm.) transverse or longitudinal sections, and placed in appropriate solutions. All blocks were introduced into the fixing solution within 1 minute after cessation of the blood flow.

For the study of lipin, tissues fixed in 1 part neutral formalin to 9 parts 0.85% NaCl for 12 to 18 hours were washed rapidly in distilled water, and embedded for 24 hours through 10%

and 20% gelatine at 37°C. Sections of the frozen material were cut at 8 μ (Hoerr, '37).

To avoid the artifacts produced by phenol and related compounds, no preservative was added to the gelatine in which the blocks were embedded; the sudan III stain used was purified by DeBruijn's method ('39). The staining solution was made by diluting a saturated 80–95% alcoholic solution of sudan III with equal parts of glycerine (Rossman, '41).

The formol-picric-alcohol mixture recommended by Rossman ('40) for the study of glycogen was also found to be satisfactory for mucoid. After fixation for 12 to 18 hours, the tissues were washed for several days in 95% alcohol, dehydrated and double embedded in celloidin and paraffin (Bensley and Bensley, '38).

Glycogen was studied after staining by one or more of the following techniques: (1) Best's carmin; (2) Bauer-Feulgen; (3) Gage's iodine (cf. Bensley, '39). In each instance the stained material was controlled by saliva-digested preparations. The Bauer and Best methods tend to stain glycogen a more brilliant red than mucus, and iodine stains glycogen a dark brown and mucus a light yellow. Still, none of these staining procedures are really specific for glycogen in the presence of mucus. The two must accordingly be differentiated by the use of saliva-digested controls.

The fixed material was controlled by a study of fresh vaginal epithelium in warm saline, or in the vaginal fluid withdrawn at biopsy.

Four zones (fig. 1) can be recognized in the vaginal epithelium of the guinea pig.

1. A superficial zone (fig. 1, A) bordering the lumen consisting of two or more layers of columnar cells with much mucoid in the cytoplasm. This zone is lost early in estrus.

2. A transitional zone (fig. 1, B) which is present only during late proestrous and estrous stages. It is composed of cells which are partially or completely "cornified" and which contain intracellular lipins.

3. A prickle-cell zone (fig. 1, C) composed of cells with spiny processes on their surfaces and with intracellular glycogen during late diestrous, proestrous, and estrous stages.

4. A basal zone (fig. 1, D) bordering the lamina propria which consists of cuboidal or columnar cells that frequently contain mitotic figures, especially during proestrus.

This is similar to the zoning used by Smith and Brunner ('35) for the human vagina. The superficial and transitional zones correspond, respectively, to the mucified and cornified layers, while the prickle-cell and basal zones are equivalent to the rete Malpighii of current usage.

Early diestrus. A section of the vaginal mucosa on the fourth proestrous day (fig. 2) shows a thin epithelium. In some areas it is but 10 to 15 μ thick and here consists of only two cell layers. Rarely only a single cell may separate the lamina propria from the lumen but this condition is never extensive enough to simulate a simple epithelium. One of the characteristic features at this time is the more or less ragged appearance of the surface. The cells bordering the lumen may be flat or elongated (fig. 2, $\downarrow$), irregularly shaped, or low and cuboidal in form. Their nuclei are compressed and oriented parallel to the long axis of the cell, and the cytoplasm stains diffusely for mucoid. However, where cuboidal cells are present in the surface layer, most of them contain small quantities of sharply stained mucoid localized apically. The cells in the basal layer of the epithelium (fig. 2, $+$) are polyhedral or columnar, their nuclei are oval and centrally located, and the cytoplasm is free of mucoid.

No glycogen or lipin can be demonstrated at this stage in the epithelium. Mitotic figures are rare. Leucocytes may be found in both the lamina propria (fig. 2, L) and the epithelium, but the extent of infiltration varies, and in some cases is minimal.

The vaginal smear made during the fourth day after estrus consists of many leucocytes and a few small nucleated epithelial cells. Mucoid material is present in the epithelial cells but lipins and glycogen are absent.

Mid-diestrus. The epithelium has increased to a height of 20 to 25 μ (fig. 3). This increase is not due to proliferation of the epithelium which is still but two layers thick. It is apparent that we are dealing, rather, with an epithelial hypertrophy resulting from an increased elaboration of mucoid in the surface cells. The majority of them are now definitely columnar in form and swollen with secretion (fig. 3, $\downarrow$). Some cells may contain little or no mucoid (fig. 3, *). There is, as yet, no evidence of glycogen or lipin, mitoses are rare, and only a few leucocytes are found infiltrating the mucosa (fig. 3, L).

The vaginal smear on the eighth day after estrus is identical with that observed on the fourth day except that the epithelial cells have diminished in number.

Late diestrus. The vagina during this phase (fig. 4) shows both proliferation and differentiation. The epithelium has reached a height of 50 to 70 μ and three zones are demarcated:

1. A superficial zone (fig. 4, A) composed of two or three layers of swollen, mucoid cells which have developed from a continued proliferation, and differentiation of the underlying epithelial cells; that is, the superficial cells do not divide.
2. A prickle-cell zone (fig. 4, C) formed by 1 to 3 cell layers.
3. A basal zone (fig. 4, D) composed of a single layer of cuboidal or columnar cells.

The cells which border the lumen contain abundant mucoid (fig. 4, $\downarrow$), which may decrease progressively as the prickle-cell zone is approached. In addition, fine granules of glycogen are dispersed throughout the cytoplasm of many superficial cells. No lipin inclusions could be demonstrated by sudan III.

Just below the superficial zone many of the cells in the prickle-cell zone contain glycogen. In most cases the glycogen is plasmolized, but a few cells show ten to twenty granules averaging 1 μ in diameter uniformly dispersed through the cytoplasm. Lipin and mucoid are absent from these cells.

The cells in the basal zone (fig. 4, D) show occasional mitoses. Selective stains failed to demonstrate glycogen, mucoid, or lipin in their cytoplasm.

No evidence of "cornification" or "keratinization" was found in the epithelium, and the leucocytic infiltration (fig. 4, L) is comparable to early diestrous stages.

The vaginal smear at this time is similar to the preceding stage, except that it frequently consists entirely of leucocytes.

Proestrus (8 to 10 hours average duration). Early during this stage, i.e., before active loss of the epithelium begins, the vagina exhibits an epithelium which is 175 to 200 μ in height in paraffin sections and is composed of 10 to 17 cell layers (fig. 5). This is the maximum thickness attained by the epithelium during any phase of the cycle. It differs from a "late diestrous" epithelium in the following respects:

1. The superficial zone (fig. 5, A) is 4 to 5 cells thick. The cells are hypertrophied owing to the increased elaboration of glycogen and mucoid. Many cells contain lipin which may also be demonstrated in the corresponding cells of the smear. In addition, fragments or clumps of hematoxylin-staining material are scattered throughout the cytoplasm. These bear no relation to the hematoxylin-staining granules described by Long and Evans ('22) which lie below the stratum corneum of estrus. The nuclei are either pycnotic or chromatolytic.

2. The prickle-cell zone (fig. 5, C) has become 8 to 10 cell layers thick. More of the cells contain glycogen (as many as twenty to thirty granules in a cell).

3. The basal zone (fig. 5, D) consists of 1 to 2 cell layers and an occasional mitotic figure can be found.

During late proestrus a sharply demarcated transitional zone (figs. 1, B and 6, B) is found between zones A and C. This new zone consists of cells (3 to 5 layers) which are greatly flattened parallel to the surface. Cells in the upper part of this zone exhibit nuclear degeneration and they contain many fine dust-like lipin droplets concentrated along the inner surface of the cell membranes. Frequently, but not always, in the deep layers of this zone hematoxylin-staining intracellular granules are seen which resemble the keratohyaline of the stratum granulosum of the epidermis.

The superficial zone (fig. 6, A) during late proestrus presents a ragged uneven surface because some cells have been shed, while others are loosened and complete desquamation appears imminent. Although the mucoid, glycogen, and hematoxylin-staining components of the cytoplasm remain comparable to "early proestrous" specimens, the lipin droplets have increased in size and number. The nuclei of all these cells show some pycnosis or chromatolysis.

The prickle-cell and basal zones remain unchanged. Mitosis and leucocytic infiltration progressively decline so that at the end of this stage dividing cells are exceedingly rare and the mucosa is free of leucocytes.

The following observations were made on transparent bits of fresh vaginal tissue removed from "proestrous" mucosae and studied in physiological media:

1. Lipin. Epithelial cells examined promptly under the oil immersion lens are found to contain highly refractile droplets which are not stained by Janus green B. Furthermore, hanging-drop preparations suspended over osmic acid vapor show that the droplets become dark in 10 to 15 minutes. These droplets agree in number and distribution with those seen in frozen sections stained with Sudan III.

2. Mucoid. The majority of cells examined immediately after removal contain many granules which are probably mucinogen, as they swell and form vacuoles which "explode" in the course of 1 to 2 minutes. Their cytoplasm then appears similar to the goblet cells of the gut. The mucoid reacts positively to Millon's reagent. When formol-picric-alcohol is added to a fresh preparation of cells their cytoplasm rapidly assumes a thready, pseudogranular structure (fig. 4, ↓) similar to mucin after it has been attacked by strong alcohol. However, unlike many mucins, the mucoid component of these cells is not soluble. It remains unaltered when thin bits of fresh tissue are hydrolyzed by 0.5% KOH or 2% HCl for 30 minutes at 37°C., then fixed in formol-picric-alcohol, digested with saliva, and stained by the Bauer-Feulgen method.

Smears made during the transition period between diestrus and proestrus reveal, in addition to the leucocytes previously present, large, oval, or irregularly-shaped epithelial cells which contain numerous vacuoles. They are obviously mucoid cells derived from the superficial zone (A) by desquamation. At first they appear singly or in small clusters. Later, the leucocytes decrease in number so that by the end of this stage, smears may consist entirely of nucleated epithelial cells. Most of the epithelial cells contain lipin; there are from 20 to 30 droplets per cell. In some cells they are from 0.2 to 0.5 μ in diameter, while in others they measure from 4 to 5 μ . Saliva digested smears stained by Bauer-Feulgen show that the cytoplasm of these cells consists chiefly of mucoid, although glycogen is present before digestion. Smears stained with Sudan III and then by the Bauer-Feulgen method demonstrate the presence of a fourth component. Clumps or fragments of material staining with hematoxylin and other "basic" dyes are scattered throughout the cytoplasm in the majority of cells. There are one to thirty clumps in a cell which vary from 4 to 0.25 μ in diameter. No apparent correlation exists, in respect to size, number, or occurrence, between the hematoxylin-stained material and the other intracellular components. Moreover, this material is never found in epithelial cells except during proestrus.

Estrus (10 to 12 hours average duration). A section of the mucosa (fig. 7) during early estrus shows the epithelium to be 110 to 120 μ thick. The epithelium has been reduced in height by the loss of the superficial cells (fig. 6, A) during the previous stage. At estrus the transitional zone (fig. 7, B) consists of 10 to 12 "cornified" cell layers and forms the free surface of the epithelium which has a ragged contour due to an irregular shedding of cells (fig. 7, $\downarrow$). The nuclei of the "cornified" cells are degenerating or do not stain. Nuclear degeneration is particularly evident in the superficial layers. No glycogen or mucoid are present in this zone but lipins are abundant, especially in the superficial layers (fig. 7, *). The lipin droplets (30 to 50 per cell) vary from small dust-like

particles that are usually arranged along the inner surface of the cell membrane to larger droplets (1 to 3 μ in diameter) scattered throughout the cytoplasm.

In the prickle-cell zone the following changes have occurred since proestrus:

1. Very few cells contain glycogen and when present, it appears in the form of small diffuse masses quite different from the granules seen in previous stages.

2. The deep layers of cells have increased in thickness despite the fact that mitotic figures are rare in the underlying basal zone.

A maximum degree of hyperemia and edema in the lamina propria and the complete disappearance of extravascular leucocytes in the mucosa are prominent features of the vagina at this time.

A study of the fresh vaginal tissue from estrous mucosae failed to demonstrate the presence of mucoid or glycogen in the "cornified" cells. However, these cells contain many, fine, highly refractile lipin droplets as in the case of the proestrous cells from the superficial zone (cf. p. 433).

The vaginal fluid at this stage contains a mass of "cornified" cells with bent and wrinkled cell membranes. In the majority, various stages of nuclear degeneration ranging from partial to complete chromatolysis are seen. Similarly, the cytoplasm shows degenerative changes, such as vacuolation and little affinity for stains. No glycogen or mucoid could be demonstrated in these cells, but Sudan III indicated that the vacuolation is due to the presence of many small lipin droplets (30 to 50 per cell) averaging 1 μ in diameter. Leucocytes are not found in the smear except during the early part of the stage, and then their presence is extremely variable.

During this stage when closely spaced vaginal samples are taken, the entire "cornified" cell layer is often recovered as perfect cast of the vaginal lumen, including even the folds about the fornices.

Metestrus (4 to 8 hours average duration). A section of the mucosa (fig. 8) during early metestrus shows the epithelium to

be 50 to 60 μ thick (8 to 10 cell layers). This reduction is due to the loss of the transitional zone (fig. 7, B) at estrus. The prickle-cells of the previous stage are now at the surface, which is very irregular, due to the presence here and there of cells that are rounding up and sloughing into the lumen (fig. 8, *). The superficial cells are free of mucoid, but contain a few, fine, lipin droplets and occasionally a small amount of glycogen. The basal zone of cells (fig. 8, D) is comparable to that of the estrus stage.

The mucosa now shows an extensive infiltration of polymorphonuclear leucocytes. As dehiscence of the surface cells proceeds, the number of leucocytes increases and they migrate toward the surface, often forming a band of infiltration just below the basement membrane. Later, the epithelium is densely infiltrated (fig. 8, L).

A section of the mucosa at the end of this stage (fig. 9) differs from an early metestrous mucosa in the following features:

1. The leucocytes, which infiltrate the epithelium, have entered the vaginal lumen in great numbers.
2. The lamina propria shows a subsidence of the leucocytic infiltration (fig. 9, L) and edema and hyperemia are reduced.
3. The epithelium is only 15 to 20 μ thick (fig. 9, $\downarrow$).

The vaginal smear early during this stage consists, typically, of small, round or oval, nucleated epithelial cells. Their nuclei appear normal and have clearly defined chromatin and nucleoli. However, the cytoplasm contains fine vacuoles and shows a wide range of affinity for stains. Specific stains reveal fine lipin droplets but no glycogen or mucoid. Occasionally a few epithelial cells may be found that differ from the cells described above in the following respects: (1) they are considerably larger; (2) their nuclei are undergoing chromatolysis; (3) and their cytoplasm contains variable amounts of mucoid. A series of vaginal smears made during this stage shows a decreasing number of epithelial cells and an increasing number of leucocytes. At the end of metestrus

the smear has returned to the essential diestrus appearance, i.e., many leucocytes and a few epithelial cells.

The subepithelial tissues are free of mucoid, glycogen, and lipin during all stages of the cycle except for the muscular layers which usually contain considerable intracellular glycogen during proestrus and estrus.

DISCUSSION

Several classifications of the vaginal smear have been proposed (Stockard and Papanicolaou, '17, '19; Selle, '22; and Young, '37) for delineating the stages of the estrous cycle in the guinea pig. In all cases these classifications have been made on the basis of specific cell types found in the vaginal fluid. In spite of the fact that they differ in some details it is obvious that the sequence of cell types desquamated is essentially as follows:

1. *Proestrus*. Relatively few leucocytes and numerous large, irregularly-shaped, nucleated, epithelial cells.

2. *Estrus*. Absence of leucocytes and many large, flattened, "cornified," squamous epithelial cells.

3. *Metestrus*. Leucocytes predominate but many small, round, nucleated epithelial cells are present.

4. *Diestrus*. Many leucocytes and a few small nucleated epithelial cells, the latter decreasing in number as the proestrous stage is approached. They differ entirely from the cell characteristic of proestrus.

This study confirms the above morphological classification but in addition makes it possible to differentiate these cell types on a basis of the substances which appear cyclically in their cytoplasm as indicated by specific staining methods (table 1, p. 438). The most distinctive feature of the proestrous smear is the appearance of large, irregularly-shaped epithelial cells which are derived from the superficial zone (fig. 5, A). Cells in this zone contain abundant mucoid, lipin, glycogen, and hematoxylin-staining material. At no other phase of the cycle do these components appear simultaneously in the cytoplasm. These intracellular components may be

selectively demonstrated in vaginal smears by staining with Sudan III and then by the Bauer-Feulgen method. This technique is especially valuable in differentiating proestrous from metestrous cells, as the latter contain only lipin, while the former contain glycogen, mucoid, and hematoxylin-staining material in addition to lipin. On the other hand, routine staining procedures (e.g., hematoxylin and eosin) show only differences in cell size. At estrus the flattened "cornified"

TABLE 1

Cyclic changes in the cytoplasm of epithelial cells from the vaginal smear.

STAGE	INTRACELLULAR COMPONENT				Characteristic cell types
	Glycogen	Lipin	Mucoid	Hematoxylin-staining material	
Proestrus	+	+	+	+	Large, irregularly-shaped, nucleated, epithelial cells Few leucocytes
Estrus	—	+	—	—	Flattened, "cornified," epithelial cells Nuclei unstained Leucocytes absent
Metestrus	—	+	—	—	Small, round, nucleated epithelial cells. Leucocytes dominant
Diestrus	—	—	+	—	Small, round or oval-shaped epithelial cells Leucocytes dominant

epithelial cells in the smear derived from the transitional zone (fig. 7, B) lack stainable nuclei and are so characteristic that this phase is easily recognized. However, the presence of abundant intracellular lipin and the lack of glycogen, mucoid, and hematoxylin-staining material provides confirmatory evidence. The metestrous smear is composed of small, round, nucleated epithelial cells desquamated from the now superficial prickle-cell zone (fig. 8, C). Although small amounts of lipin are found in their cytoplasm, none contain lipin in

amounts comparable to the "cornified" cell of estrus. In addition, many metestrous cells lack intracellular lipin. Mucoid, glycogen, and hematoxylin-staining material are absent. Epithelial cells of the diestrous smear are derived from the proliferating surface epithelium (fig. 2). They do not contain intracellular glycogen, lipin, or hematoxylin-staining material, but mucoid is present and this increases progressively as the proestrous stage is approached.

The characteristic function of mucous cells is to elaborate a secretion which is usually a lubricant and this may be the function of the mucoid cells of the vaginal epithelium. They reach their maximum development during proestrus (fig. 5, A) and during the final weeks of pregnancy (fig. 10, A). Mucus might well facilitate copulation and parturition. It would not be necessary for these cells to discharge the mucus as was postulated by Lelievre and Retterer ('10), for a readily detached layer of "exploded" mucous-laden cells might be an excellent lubricant. Kelly ('29) has emphasized the stability of the desquamated mucous cell in the vagina of the pregnant guinea pig. The free epithelial cells in the proestrous vaginal fluid are likewise stable, i.e., they are highly resistant to alkaline or acid hydrolysis and show no signs of autocytolysis or leucocytolysis. Nevertheless, mucoid may be demonstrated in the vaginal fluid by selective stains, e.g., mucicarmin, muchematein or the metachromatic reaction. That this mucus is not derived entirely from uterine and cervical secretions has been demonstrated by Selle ('22) who found vaginal mucus present, although reduced in quantity, in guinea pigs from which the uterus and cervix had been removed. This definitely indicates that the superficial vaginal cell elaborates and secretes at least part of the mucoid component of the vaginal fluid.

The occurrence of intracellular glycogen and lipin in the superficial zone of the "proestrous" vagina may indicate merely cellular depression. These constituents accumulate rapidly during this time and reach a maximum concentration just before the onset of the massive desquamation of the

estrous phase. As the transitional zone (fig. 6, B) differentiates prior to desquamation, a condition exists in the epithelium which might lower the metabolism of the superficial cells. The transitional zone may act as an effective tissue barrier. Since the epithelium is dependent upon a slow diffusion through the intercellular spaces which are greatly reduced here, the cellular exchange of metabolites in the superficial zone would be greatly retarded or possibly blocked. Moreover, even before the intercellular fluid reaches the transitional zone it must diffuse through 10 to 12 cell layers of the prickle-cell and basal zones (fig. 6, C and D). In addition to the altered diffusion, the tissue fluid itself would be inadequate for cellular respiration, since the anabolic gradient would be depressed before the fluid reached the level of the superficial cells. Such a mechanism has been suggested by Cowdry ('32) as one of the factors involved in the keratinization of the epidermis.

Clark and Clark ('40) in their study of transparent chambers in the rabbit ear have reported that fluctuations in the blood supply to subcutaneous tissue were an important factor in lipin formation. They found that lipin formation occurred only in chambers that had a moderate or slow circulation. After new lipin had formed, it showed a tendency to diminish during or immediately following a period of active circulation and to increase again with the return of a reduced circulation.

A reduction of the blood supply may likewise account for the accumulation of intracellular glycogen. Gierke ('05) found much glycogen after ligation of the renal artery in the epithelium adjacent to the infarcted area.

In any case, when the epithelium has reached its greatest thickness at proestrus, there are mucoid, glycogen, lipin, and hematoxylin-staining material in the superficial zone. Since mucoid appears in minimal amounts in the epithelium at diestrus, and gradually accumulates as the follicles mature, there is good reason for assuming that the increase in mucoid is a specific response to a progressive increase in the output

of estrogen. The desquamation of these cells at the time of estrus, and the evidence that they secrete some of the mucoid found free in the vaginal fluid, can be interpreted as a functional adaptation for coitus. On the other hand, lipin droplets can not be so readily explained. They appear in the superficial cells rather rapidly, their appearance heralding the imminent onset of sexual receptivity. There is no evidence that they are liberated into the secretion. Their presence in a layer of cells which at this time is farthest removed from the blood supply, and their coming at the end of a cycle of mucoid secretion, suggests rather that we are dealing with a regressive process of lipophanerosis. It is well-known from many studies of pathological changes in cells under the influence of ischemia, that lipins may appear in the cytoplasm.

Glycogen in the vaginal fluid at estrus might have some functional significance for the survival of spermatozoa. Nevertheless, its appearance may be due to the development of a slow depression in metabolic activity. This is the cycle of events seen in many carcinomatous growths, which are apt to outgrow their blood supply. A similar sequence of events was observed by Rossman ('40) in the case of the epithelial proliferation in macaque deciduomata; here the accumulation of glycogen was followed by the appearance of lipin droplets and shortly thereafter by frank necrobiosis. A similar interpretation may be made of the secretory cycle in the primate uterine gland cell (cf. Rossman, '41). Here again, towards the close of a cycle of mucoid secretion, lipins appear in increasing amount. The functional significance of these changes in the uterus from the viewpoint of embryotrophe is clear, although the changes may be regarded as regressive in terms of the life-cycle of the cell. A similar interpretation may be made in the case of the vaginal epithelial cells, so that we may conceive of the epithelium of the proestrous stage as representing the end of a progressive phase of cell activity — elaboration of mucoid — coupled with the beginning of a regressive phase as indicated particularly by the appearance of lipin droplets in the same cells.

The accumulation of intracellular lipin in the transitional zone at proestrus and estrus and the presence of intracellular glycogen in the prickle-cell zone during late diestrus and proestrus may be explained by one or more of the mechanisms discussed above. On the other hand, glycogen, mucoid, lipid, or hematoxylin-staining material are never found in the basal zone at any stage of the cycle.

The nature of the intracellular hematoxylin-staining material found only during proestrus in cells of the superficial zone has not been thoroughly investigated. However, it gives a negative Feulgen reaction and is not affected by common fixing reagents. In these respects it is similar to the basophil substance described by Bartelmez and Bensley ('32) in uterine gland cells.

Regeneration of the epithelium takes place from the persisting cell layers of the basal zone, after the estrous desquamation. In this connection it should be emphasized that the vagina is never covered with a simple columnar epithelium as Kelly supposed. The paucity of mitoses recognizable in the vaginal epithelium during the rapid growth phase (proestrus) has long been a problem which has been recently clarified by Allen, Smith and Gardner ('37). They found that large numbers of mitotic figures had accumulated in the hyperplastic vaginal epithelium when colchicine was injected into estrogen primed mice about 10 hours before death.

Stockard and Papanicolaou ('17) reported that the epithelial destruction at estrus may extend down to the lamina propria and that slight hemorrhages may occur into the vaginal lumen from the congested capillary bed. In contradiction to this, we can corroborate Selle's ('22) observations that the lamina propria is always covered by epithelial cells and that signs of hemorrhage were rarely (in our experience never) present in the vaginal fluid examined at this stage. Vaginal hemorrhage may well be due to injury of the capillary bed by instrumentation.

SUMMARY

1. The cyclic changes in the vaginal mucosa, and particularly the mucoid, glycogen, and lipin constituents in the epithelium, of the guinea pig have been studied by cytological techniques and correlated with changes in the vaginal smear.

2. On the basis of the present findings four epithelial zones can be recognized which are not, however, present in all stages of the estrous cycle.

a. A superficial zone bordering the lumen.

b. A transitional zone whose cells are partially or completely "cornified."

c. A prickle-cell zone.

d. A basal zone bordering the lamina propria.

3. Variations in the intracellular mucoid, glycogen, lipin, and hematoxylin-staining material of vaginal smear cells are an adequate index for some of the cytological processes occurring in the vaginal epithelium. Therefore, the vaginal smear, when adequately stained, may be classified on a basis of cell types which are indicated by their specific components.

4. Intracellular mucoid is restricted to the superficial epithelial cells and its elaboration is maximal during the proestrous stage. These cells probably secrete at least part of the mucoid component of the vaginal fluid.

5. Intracellular glycogen reaches a maximum concentration during the proestrous stage and is present in the superficial and prickle-cell zones.

6. Intracellular lipin is most abundant during the proestrous and estrous stages and is found in the superficial, transitional, and prickle-cell zones.

7. Intracellular hematoxylin-staining material is found only during the proestrous stage and in the superficial zone.

8. The subepithelial tissues do not contain mucoid, lipin, or hematoxylin-staining material, and no pronounced cyclic changes in the glycogen content have been observed. Variable amounts of intracellular glycogen can be found in the muscle layers of some proestrous and estrous specimens.

9. The possibility has been discussed that the occurrence of lipin during proestrus and estrus and the high glycogen content at proestrus may indicate a cellular depression.

10. Cellular desquamation, during the metestrous stage, reduces the epithelium to two or more layers of cells and it is from these persisting basal layers that regeneration takes place.

I am indebted to Dr. I. Rossman for critical advice kindly extended during the course of this study, and to Mr. R. D. Bensley for the photomicrographs. I should like to express at this time my thanks to Prof. G. W. Bartelmez, under whose direction this study was performed.

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PLATES

EXPLANATION OF PLATES

Figure 1 is a camera lucida drawing at 970 diameters reduced to 400 diameters. Figures 2-10 are from photomicrographs taken with a 3.5 mm. apochr. imm. lens and a X 10 ocular at 400 diameters.

PLATE 1

EXPLANATION OF FIGURES

1 Vaginal mucosa during late proestrus: A, superficial zone; B, transitional zone; C, prickle-cell zone; D, basal zone; E, lamina propria; $\uparrow$ mitotic figure; + vacuolated cytoplasm which is typical when cells are not stained selectively for mucoid. 4 μ section, fixation formol-picric-alcohol, stained by Shorr's trichrome method.

2 Vaginal mucosa 4 days after estrus. The surface is irregular in contour and consists of three layers of cells at its thinnest portion (+). Cells bordering the lumen at $\downarrow$ are elongated, their nuclei compressed, and their cytoplasm stained diffusely for mucoid. The basal cells at + are polyhedral, their nuclei oval, and the cytoplasm is free of mucoid. Leucocytes are found in the lamina propria and epithelium at L. 4 μ section, fixation as in figure 1, and stained by the Bauer-Feulgen method.

3 Vaginal mucosa 8 days after estrus. The epithelium shows no increase in the number of cell layers when compared with 4-day specimens. However, cells bordering the lumen are cuboidal or columnar and swollen by mucoid ($\downarrow$). In some cells (*) little or no mucoid is present. The basal cells at + are free of mucoid. Leucocytes are present in the epithelium and lamina propria at L. 4 μ section, fixation and staining as in figure 2.

4 Vaginal mucosa 12 days after estrus. The epithelium consists of three zones: A, a superficial zone which is composed of two to three layers of swollen columnar cells that contain a large amount of mucoid ($\downarrow$). Cells at $\downarrow$ show the typical pseudogranular appearance of mucoid after formol-picric-alcohol fixation. Cells deeper in this layer may contain but little mucoid as at +; C, prickle-cell zone which is formed of one to three layers and resembles the stratum spinosum of the epidermis; D, a basal zone composed of cuboidal or columnar cells. Leucocytes are found in the lamina propria at L. 4 μ section, fixation and staining as in figure 2.

5 Vaginal epithelium during early proestrus. The superficial zone (A) is composed of 4 to 5 cell layers and the intracellular mucoid is similar to that found in the 12-day specimens (cf. fig. 4) in quantity and distribution. The prickle-cell zone (C) is 8 to 10 cell layers in thickness. Most of the prickle cells contain glycogen (*) which appears black, and in greater amounts than in similar cells of the 12-day specimens. Leucocytes appear in the epithelium and lamina propria at L. 4 μ section, fixation and staining as in figure 2.

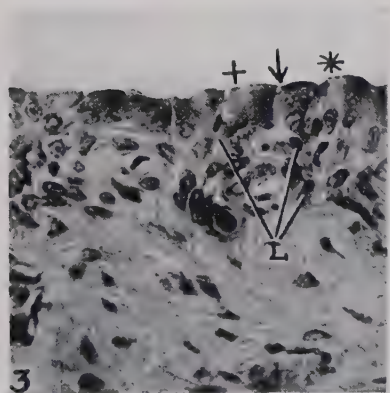
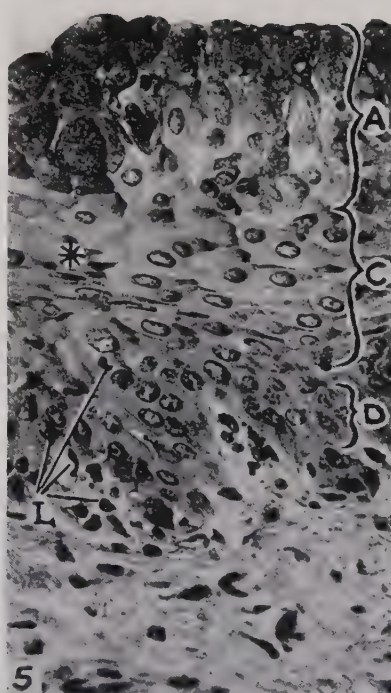
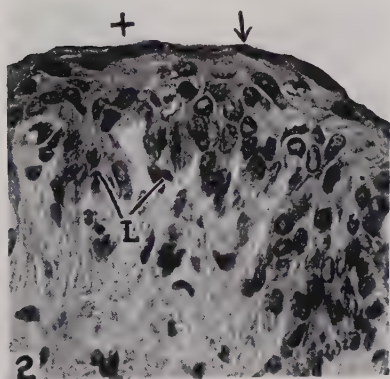
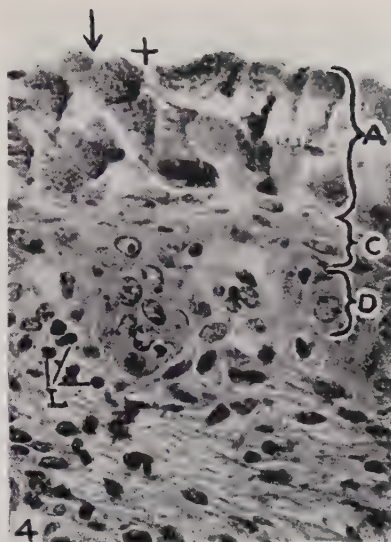
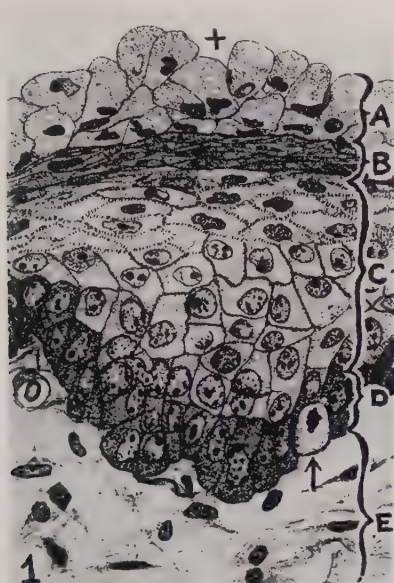


PLATE 2

EXPLANATION OF FIGURES

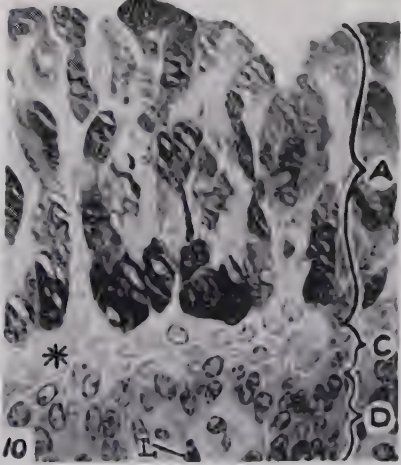
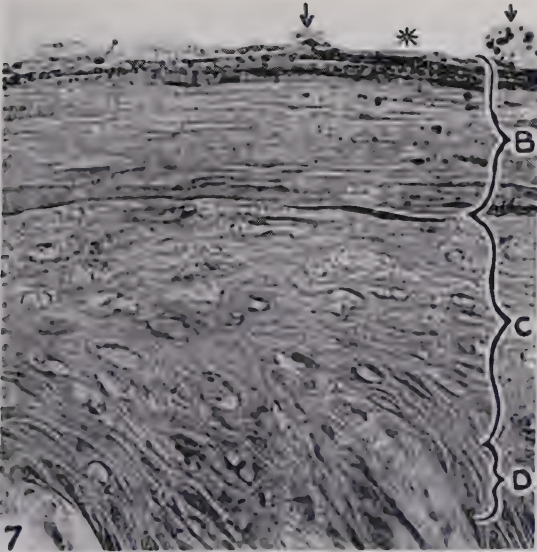
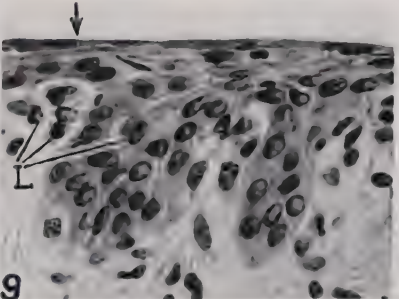
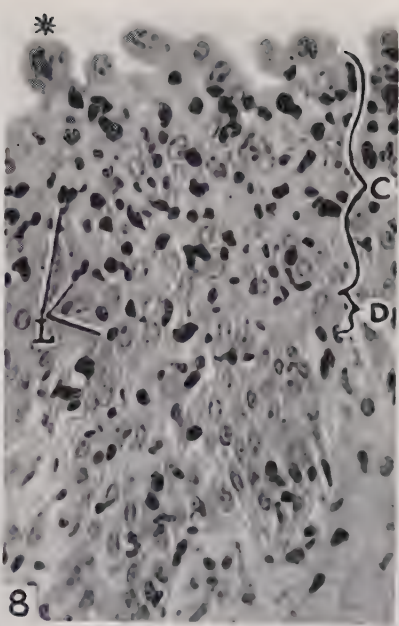
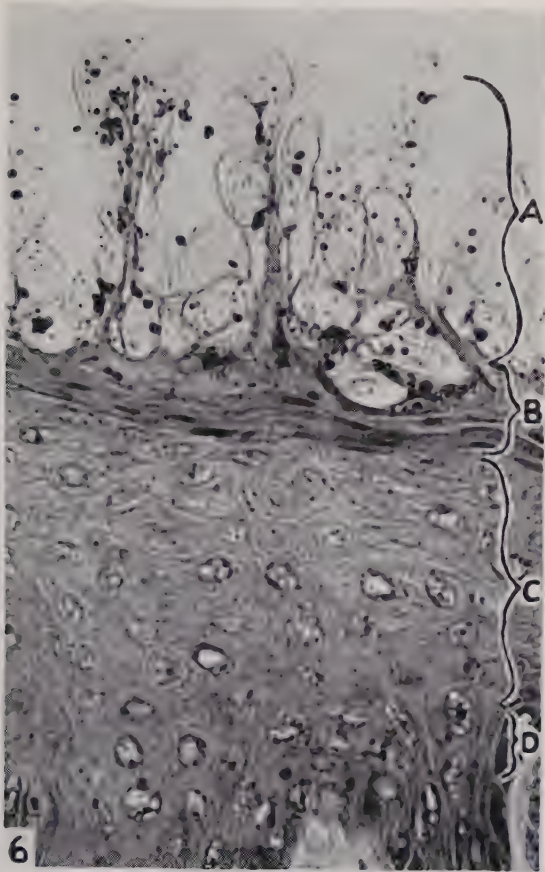
6 Vaginal mucosa during late proestrus. From the same mucosa as that shown in figure 1. The lipin droplets in the cells of the superficial zone (A) appear black. Intracellular mucoid is not stained in this preparation but the material stained by the Bauer-Feulgen method demonstrates a large amount of this component. The transitional zone (B) consists of 3 to 5 cell layers which are undergoing "cornification." Most of the "cornified" cells contain fine, dust-like, lipin droplets concentrated along the inner surface of the cell membranes. The prickle-cell zone (C) is 8 to 10 cell layers in thickness and the cells are free of intracellular lipin. Mitoses and leucocytes are absent in the epithelium and lamina propria. 7 μ frozen section, fixed in 10% formol in saline, stained by Sudan III, and mounted in glycerine. In this and in figure 7 there is much less shrinkage than in the paraffin sections.

7 Vaginal mucosa during estrus. Complete desquamation of the superficial zone has taken place and the transitional zone (B) forms the free surface. Here the cells are typically "cornified." They contain many lipin droplets. Cells at ↓ are in the process of desquamation. The lipin is particularly abundant in cells of the superficial fourth of the transitional zone (*). The prickle-cell zone (C) is identical with the similar zone of late "proestrus" specimens (cf. fig. 6), except that it consists of fewer cell layers. Mitoses and leucocytes are absent in the lamina propria and epithelium. 7 μ frozen section, fixation and staining as in figure 6.

8 Vaginal mucosa during early metestrus. Desquamation has progressed to the upper cell layer of the prickle-cell zone (C). The cell at the surface (*) is "rounding up" preparatory to sloughing and contains a small amount of glycogen. Leucocytes have extensively infiltrated the epithelium and are migrating through it (L). No mitoses are present. 4 μ section, fixation formol-picric-alcohol, and stained by the Bauer-Feulgen method.

9 Vaginal mucosa during late metestrus. Massive desquamation is over. The epithelium presents a smooth surface and consists of 3 cell layers at ↓. A marked reduction in the degree of the leucocytic infiltration has obviously taken place (cf. fig. 8). Only a few leucocytes are present in the epithelium and lamina propria (L). No mitoses are present. 5 μ section, fixation and staining as in figure 8.

10 Vaginal mucosa during the sixth week of pregnancy. The superficial zone (A) consists of 4 to 5 cell layers and all the cells in this zone contain large amounts of mucoid. There is no evidence of a transitional zone. The cells (*) of the prickle-cell zone (C) have a faintly stained vacuolated cytoplasm. Cells in the basal zone (D) are identical to similar cells in "proestrus" mucosae (cf. fig. 5). Leucocyte in the lamina propria at L. 5 μ section, fixation aqueous chrom-sublimate (Bensley), and stained by the Bauer-Feulgen method.



APPROVED

MOTION PICTURE FILMS

The following films have been approved by one or more members of the Review Committees of the American Association of Anatomists or the American Society of Zoologists, and The Wistar Institute:

SUBJECT AND TITLE	LENGTH IN REELS (1-16 MM. REEL = 400 FT. RUNNING TIME ABOUT 15 MIN.)	PRODUCER	CONDITIONS FOR SECURING FILMS	DISTRIBUTOR
ANATOMY				
Congenital anomalies (monochrome, silent) (kodachrome, silent)	1	J. Sarnoff	Sale: Monochrome, \$25.00 Kodachrome, \$75.00	Sarnoff Motion Picture Film Library 1406 Albemarle Road Brooklyn, N. Y.
* The management of cica- tricial strictures of the esophagus. Treatment of cancer of the esopha- gus. Bronchoscopy with insufflation of sulfanil- amide in chronic bronchiectasis (kodachrome, silent)	1	E. H. Ingersoll	Rental, \$8.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* Surgical anatomy (kodachrome, silent)	4	C. J. Baumgartner	Sale Kodachrome	Billy Burke Productions 7416 Beverly Blvd. Hollywood, Calif.
BLOOD CELLS				
* 10. Normal and abnormal white blood cells in tissue cultures (monochrome, silent)	1	W. H. Lewis M. R. Lewis A. R. Rich M. M. Wintrobe	Rental, \$4.00 per day Sale, \$25.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
CANCER CELLS				
* 6. Dividing cancer cells in vitro (monochrome, silent)	160 ft.	W. H. Lewis M. R. Lewis	Rental, \$2.00 per day Sale, \$10.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* 3. Tumor cells and macro- phages in tissue cul- tures. Rat sarcomas and carcinomas (monochrome, silent)	360 ft.	W. H. Lewis	Rental, \$3.50 per day Sale, \$20.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
CIRCULATION				
* The control of small blood vessels (mono- chrome, silent)	1	G. P. Fulton B. R. Lutz	Rental or sale	Dr. G. P. Fulton College of Liberal Arts Boston University Boston, Mass.

SUBJECT AND TITLE	LENGTH IN REELS (1-16 MM. REEL = 400 FT. RUNNING TIME ABOUT 15 MIN.)	PRODUCER	CONDITIONS FOR SECURING FILMS	DISTRIBUTOR
<i>Circulation (Cont.)</i>				
* Effect of various drugs on contractions of mesenteric lymphatics in the rat (monochrome, silent)	300 ft.	R. L. Webb	Rental, \$3.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* The heart and circulation (monochrome, silent)	1	A. J. Carlson	Sale	Erpi Classroom Films, Inc. 35-11 35th Ave. Long Island City N. Y.
* Mesenteric lymphatics, their conduct and the behavior of their valves in the living rat (monochrome, silent)	1	R. L. Webb	Rental, \$4.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* Micromanipulative studies of blood capillaries (monochrome, silent)	1	B. W. Zweifach	Rental, \$4.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* 7. Growth of capillaries and endothelium from the sub-cutaneous tissue of 7-day chick embryos in 2-day cultures in Locke-bouillon-dextrose medium (monochrome, silent)	140 ft.	W. H. Lewis	Rental, \$2.00 per day Sale, \$9.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
CYTOLOGY				
* 8. Pinocytosis. Drinking by cells (monochrome, silent)	264 ft.	W. H. Lewis	Rental, \$3.00 per day Sale, \$16.50	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* 9. Dividing normal adult rat fibroblasts in vitro (monochrome, silent)	160 ft.	W. H. Lewis	Rental, \$2.00 per day Sale, \$10.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
EMBRYOLOGY				
* Activity of heart muscle, from rat embryos, grown in tissue culture (monochrome, silent)	1	C. M. Goss	Rental, \$4.00 Sale, \$25.00	Dr. C. M. Goss University of Alabama University, Ala.
Development of a salamander (monochrome, silent)	1	L. S. Stone and T. C. Kramer	Rental Sale	Films, Inc. 330 W. 42nd St. New York, N. Y.
* 1. The early development of the rabbit egg in vitro (monochrome, silent)	1	W. H. Lewis and P. W. Gregory	Rental, \$4.00 per day Sale, \$25.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* 4. The early development of the rabbit egg (short film — selected scenes from the long film) (monochrome, silent)	80 ft.	W. H. Lewis and P. W. Gregory	Rental, \$1.50 per day Sale, \$5.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.

SUBJECT AND TITLE	LENGTH IN REELS (1-16 MM. REEL = 400 FT. RUNNING TIME ABOUT 15 MIN.)	PRODUCER	CONDITIONS FOR SECURING FILMS	DISTRIBUTOR
<i>Embryology (Cont.)</i>				
* 5. Early divisions, 2-8 cells, of living monkey egg in vitro (monochrome, silent)	80 ft.	W. H. Lewis and C. G. Hartman	Rental, \$1.50 per day Sale, \$5.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
Forming an egg (kodachrome, sound)	1	D. C. Warren and H. M. Scott	Rental, \$3.00 per day	Poultry Department Kansas State College Manhattan, Kans.
* Where chick life begins (kodachrome, silent)	1	A. L. Romanoff	Rental Sale	Ralston Purina Co. St. Louis, Mo.
* 11. Development of Zebra fish egg (monochrome silent)	1	W. H. Lewis and E. C. Roosen-Runge	Rental, \$4.00 per day Sale, \$25.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* Development of the opossum (kodachrome silent)	150 ft.	E. J. Farris	Rental, \$3.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
GENERAL				
Practical preventive orthodontics (kodachrome silent)	2	M. J. Futterman	Rental	Dr. M. J. Futterman 1749 Grand Concourse Bronx, N. Y.
* High speed motion pictures taken with stroboscopic light (monochrome, silent)	1	H. E. Edgerton K. J. Germeshausen H. E. Grier	Rental free Sale, \$25.00	Prof. Charles E. Locke Massachusetts Institute of Technology Cambridge, Mass.
* Seeing the unseen (monochrome, silent)	1	H. E. Edgerton	Rental free Sale, \$25.00	Prof. Charles E. Locke Massachusetts Institute of Technology Cambridge, Mass.
* Tropical opossums (kodachrome, silent)	1	E. J. Farris	Rental, \$8.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* Unseen worlds (monochrome, sound)	1	RCA Victor Camden, N. J.	Transportation charges only	Wm. J. Ganz Co. 19 E. 47 St. New York, N. Y.
NERVOUS SYSTEM				
* Effect of electrical stimulation of the right ansiform lobe of the cerebellum of the cat (<i>Felis domestica</i>) (monochrome, silent)	100 ft.	S. L. Clark	Rental	Dr. Sam L. Clark Department of Anatomy Vanderbilt University Nashville, Tenn.
* The eyes of science (monochrome, silent)	3	Bausch & Lomb Optical Co.	Rental free	Bausch & Lomb Optical Co. Rochester, N. Y.
* The nervous system (monochrome, silent)	14	I. V. Pavlov and others	Rental or sale	Brandon Films, Inc. 1600 Broadway New York, N. Y.

SUBJECT AND TITLE	LENGTH IN REELS (1-16 MM. REEL = 400 FT. RUNNING TIME ABOUT 15 MIN.)	PRODUCER	CONDITIONS FOR SECURING FILMS	DISTRIBUTOR
REPRODUCTION				
* The biology of reproduction in rats (kodachrome, silent)	1	E. J. Farris	Rental, \$8.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* Maternal behavior in the rat (monochrome, silent)	350 ft.	F. A. Beach	Rental, \$8.00 per day Sale, \$30.00	American Museum of Natural History New York, N. Y.
Mating behavior in the leopard frog, <i>Rana</i> <i>pipiens</i> (monochrome, silent)	250 ft.	F. A. Beach	Rental, \$8.00 per day Sale, \$20.00	American Museum of Natural History New York, N. Y.
Ovulation in the frog, <i>Rana pipiens</i> (mono- chrome, silent)	512 ft.	R. Rugh	Rental, \$5.00 per day Sale, \$25.00	Dr. Roberts Rugh N. Y. University Washington Square College New York, N. Y.
* Proestrous and oestrous behavior in the guinea pig (monochrome, silent)	150 ft.	W. C. Young E. W. Dempsey R. Hertz and H. L. Myers	Rental, \$1.50 per day Sale	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
A rapid pregnancy test (kodachrome, silent)	250 ft.	U. J. Salmon and others	Rental free	Dr. U. J. Salmon 875 Fifth Avenue New York, N. Y.
* Studies in human fertility	1 (2000 ft.)	Audio Produc- tions, Inc.	Rental free	Ortho Products, Inc. Linden, N. J.
RESPIRATION				
Mechanisms of breathing (monochrome, sound)	1	V. Johnson	Sale	Erpi Classroom Films, Inc. 35-11 35th Ave. Long Island City N. Y.

* Copy deposited in the Film Depository of The Wistar Institute, available to all scientists for use at The Wistar Institute.

MULTIPLE CONGENITAL ANOMALIES IN A STILLBORN INFANT

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TWO TEXT-FIGURES AND ONE PLATE (THREE FIGURES)

The monstrosity with which the present study is concerned presented the following eighteen anomalies: Inferior gnathoschisis, bifid tongue, anophthalmia, cleft face (including cleft nose), harelip, cleft palate, acrania, anencephalic masses, amniotic adhesions, adherent placenta (fused to skull), right clubfoot, pendulous erupted incisor tooth, absent parotid glands, aberrant thymus tissue on surface of neck, misplaced innominate vein (anterior to clavicle and sternocleidomastoideus), absence of pendant part of great omentum, atrophic ovaries, and free zygomatic bones.

INTRODUCTION

An investigation of the literature discloses no case of multiple anomalies comparable to the one herein reported. Of the various deformities presented, those of the head and face seemed most significant and will therefore be given primary consideration.

According to Davis ('35) and Ivy ('37) harelip and cleft palate are the commonest of all congenital deformities of the facial region, but, in spite of this, cleft defects so severe as to extend all the way from the chin to the cranial vault have apparently not hitherto been encountered. Murphy ('40) commented on the high frequency of harelip and cleft palate and remarked that clefts of the nose, lacrimal duct, cheek, orbit,

and forehead are not particularly rare. Cleft mandible (inferior gnathoschisis) and bifid tongue (schistoglossia), however, are very uncommon. We have found only eleven published reports of mandibular cleft in man and thirteen of bifid tongue. Each of these differed from the present case in that none was associated with such extensive clefts or with other anomalies of the face and head.

While the case to be described in this paper represents the fourteenth report of inferior gnathoschisis it is but the sixth known to be associated with a bifid tongue. Greig ('25) concluded that schistoglossia is usually secondary to more extensive malformations of the lower lip and mandible. Cleft tongue, either bifid or trifid, and cleft mandible are about equally rare. Only eight reports of schistoglossia, excluding inferior gnathoschisis, have been noted in the literature to date.

Congenitally erupted teeth are seldom reported. Although we have been able to find reference to but six cases since 1900, it is probable that the anomaly occurs more frequently than this figure suggests. However, no record of a pedunculated tooth seems to have been published hitherto. Gunson ('14) concluded from maternity statistics that the incidence of congenitally erupted teeth is about 1:5000. He quoted Kellock to the effect that this anomaly is frequently associated with harelip and cleft palate and therefore probably not the result of a syphilitic infection. Weisman's case ('38) was the only one of the six reported since 1900 in which there were associated congenital anomalies and these were less extensive than those in the present case.

Attachment of the placenta to the fetal head has been reported only eighteen times in the past 117 years. Bogatsch (1882) reviewed the literature covering fifty cases of abnormal attachment of fetus and afterbirth. He listed no less than eleven instances in which the placenta was attached to the infant's head. His references dated back as far as 1826. Since 1899 but seven additional reports of this condition have appeared making the present case the nineteenth of conjoined

head and placenta. It is a striking fact that in every published case of this extraordinary affiliation there were also present multiple congenital malformations. This is what one might expect in the light of Streeter's discussion of the ontogenesis of cephalo-amniotic anomalies (see below under "discussion").

The number of reported cases of anophthalmia is so large that this condition can hardly be designated as "rare." As to incidence, Davis ('35) found one case of anophthalmia in 1000 congenital deformities of the face. According to Rosenbaum ('31) anophthalmia varies in severity from a complete absence of ocular ectodermal structures (very rare) to simple microphthalmia. Rosenbaum quoted Ida Mann's explanation for the rarity of the most severe cases, stating that growth aberrations gross enough to completely suppress the front of the forebrain at or near the 7.5 mm. stage are liable to result in the embryo's death. In this connection it is indeed remarkable that the monstrosity with which this report is concerned lived to within 2 weeks of full term. It is apparent from a review of the literature that such optic dysplasias frequently appear in combination with deformities in both brain and skull.

OBSERVATIONS

The monstrosity herein described was a female, delivered 2 weeks prematurely. Superficial examination at birth revealed a condition of generalized cutaneous sloughing and a darkened umbilical cord, both indicative of death some days prior to parturition. After formalin fixation the infant weighed 1470 gm. and the placenta, which measured 12.4 by 11.6 by 3.5 cm., weighed 304 gm. Further examination disclosed a grossly malformed fetus which presented eighteen anomalies as listed at the beginning of this paper. These will now be described.

Lower lip, jaw, and tongue. The right and left halves of the lower lip and jaw were widely separated exhibiting a bifid tongue in the space between their exposed margins. The cleft



Fig. 1 Anterior view of monster showing most of the anomalies which were visible externally. The umbilical cord was displaced posteriorly so as not to obstruct the view of the craniofacial defects. The severed fetal end of the umbilical cord appears just above the right knee: 1, umbilical cord; 2, 2', placenta showing amniotic surface; 3, two of the three anencephalic masses; 4, amniotic adhesive bands; 5, displaced nasal structures; 6, 6', hypoplastic eyelids; 7, bifid tongue seen through mandibular cleft; 8, pendant erupted incisor tooth; 9, darkened lower extremity of amniotic adhesions; 10, aberrant thymus tissue; 11, necrotic area of unknown etiology; 12, clubfoot. $\times 0.37$.

in the tongue measured 1 cm. in depth anteriorly and continued posteriorly over its dorsum as an increasingly shallower groove to its termination in the root of the tongue. A fully erupted incisor tooth hung from the left half of the mandible by a stalk which measured approximately 1 cm. in length (fig. 1). Unerupted deciduous teeth were present in the alveolar processes of both upper and lower jaws. Beneath the mandible



Fig. 2 Left side of fetus showing direct attachment of placenta to head through amniotic adhesions. Note the cicatricial tissue extending from the amniotic adhesions to the neck and mandible. $\times 0.37$.

and in the region of the neck a broad blackened cicatricial band (apparently amniotic) was seen which averaged 2.5 cm. in width as it passed the left ear posteriorly and fanned out dorsally on the fetal surface of the cranially adherent placenta (fig. 2).

Anophthalmia. The eyes were mere slits bordered by hypoplastic lids, those on the left being devoid of lashes while those on the right were more nearly normal and bore lashes

(figs. 1 and 3). On each side eyeballs were lacking except for an unorganized, slightly differentiated mass which had fused to the lids superficially. Microscopic examination of these tissue masses revealed scattered bits of sclera, choroid, retina, and extraocular voluntary muscle. No lens tissue was found (figs. 3 and 4). The tissue contained hematoidin burrs, hemosiderin granules, and psammoma bodies (calcareous concretions). The two former were considered as indicative of early embryonic hemorrhage and imperfect blood circulation in this region while the latter suggests that lipoidal degeneration was in progress prior to the infant's death. The hemorrhagic area had been largely replaced by fibroblasts but numerous erythrocytes were present in the vicinity of rupture between the two main masses of retinal fragments (fig. 4).

Cleft face, nose, maxilla and palate. The face presented a complete vertical cleft measuring 4 cm. in length and 2.5 cm. at its greatest width. While it was medial in the mandible it extended paramesially through the maxilla, right nasal cavity and orbital portion of the frontal bone to the rim of the open skull. The maxillary cleft passed between the right maxilla and the right premaxilla. The latter was fused with its mate on the left side. The skin of the face continued to within 1 cm. of the cleft on either side except at the mouth where this distance was increased to 2 cm. The mouth was located about midway between the ends of the cleft and its communication with the pharynx was normal. Above the mouth at the edge of the skull cavity the cleft was about $\frac{1}{2}$ cm. deep and this depth increased progressively as the cleft neared the mouth where it continued backward in the palatine bone as far as the anterior pillars of the fauces resulting in a right cleft palate (fig. 1).

Just above the tongue was a bilateral pair of firm, dark, deltoid masses, the left one of which was the larger. This was joined above to the meninges by an amniotic band (fig. 1). The right mass contained the right maxilla minus its premaxillary portion while the left mass contained the complete left maxilla plus the right premaxilla. This wide separation

of maxilla and premaxilla on the right resulted in a severe harelip located just to the right of the midline. A palatine process directed vertically was also present on the left maxilla. The corresponding right palatine process was lacking. These bones were distorted in such a way as to cause the medial border of the left maxilla to be directed upward and to the left instead of toward the corresponding border of the right maxilla with which it would normally have fused in the midline. The right premaxilla lay just above this upturned mesial border of the left maxilla. The base of the former was directed vertically instead of horizontally.

Skull, placenta and amniotic bands. Examination of the skull disclosed an absence of both parietal bones except for a narrow strip of the one on the right. The squama of the frontal bone was lacking and the free upper border of the occipital bone was involuted for a distance of about 3 mm. Both temporal and occipital bones were irregular in shape. The floor of the skull cavity, though complete, was distorted, its anterior portion appeared as though having been shifted to the left. Above the margins of the skull the skin and dura apparently blended forming a slightly darkened, "leathery" layer which covered three anencephalic masses or meningeal sacs. In the lower edge of this layer were several small nodules of bone, perhaps fragments of the largely absent parietals.

A band of amnion extended from the superior end of the facial cleft across the left anterolateral portion of the anencephalic masses to a point just posterior to the left ear where it joined the cicatricial band before mentioned that passed from this point to the base of the mandible. The placenta was bound to the left anterolateral region of the skull by the bands mentioned above and by other similar amniotic adhesions which extended between the placenta and the meningeal sacs (figs. 1 and 2). The umbilical cord joined the placenta marginally near the midline and just anterior to the major portion of the anencephalic masses. Both placenta and cord were grossly normal in appearance except for obvious post-mortem change. Microscopic examination of the placenta

showed no pathological changes except for the presence of hemosiderin which was verified by the Prussian-blue reaction. This finding of hemosiderin in the placenta suggests, as in the case of the orbitopalpebral tissues, the presence of an old hemorrhage together with faulty circulation in that part. This condition may have been significant as a factor in the causation of prenatal death in the present case.

Anencephalic masses. The placenta and amniotic adhesions were so attached to the infant's head as to constrict the cranial contents into three encephalocele-like masses. The largest of these was on the right side of the head (fig. 1), the middle-sized one lay just above and behind the left ear (fig. 2) while the smallest was somewhat flattened and partially overlay the nose, being situated above and to the left of the facial cleft (fig. 1). Each of these masses consisted of a double-layered meningeal sac, the largest of which contained bits of nearly decomposed cerebral tissue. The smaller ones contained nothing but unrecognizable gelatinous material.

Thymic tissue. A pedunculated tag of aberrant thymus tissue was attached superficially to the left side of the neck. It was histologically verified (fig. 5).

Clubfoot. Gross examination of the lower limbs indicated the presence of a right clubfoot. This diagnosis was confirmed by roentgenograms.

Absence of venereal disease. Kolmer and Kline tests of the blood of the monstrosity's mother were both negative for syphilis. Although she had been suspected of once having had gonorrhea, two vaginal smears taken just prior to the infant's birth showed no gram negative intracellular diplococci.

DISCUSSION

Numerous theories relating to the etiology of congenital anomalies have been proposed but today few if any of the older explanations are accepted. In general, extrinsic hypotheses are difficult to prove, consequently such views are now seldom advocated. Modern teratologists tend to favor intrinsic or hereditary theories of teratogenesis. Nevertheless, the careful

observer will ever be on the look-out for positive evidence of any infectious organism such as *Treponema pallidum* which, acting in utero or otherwise, might be a factor in monster formation. With this in mind the authors desire to emphasize that laboratory tests for syphilis and gonorrhea were negative in the case reported in this article. The mother, a 21-year-old primipara, was apparently healthy and gave no history of operations or serious accidents. She reported having had tuberculosis, measles, and scarlet fever during childhood. Interestingly enough, her sister had been thrice pregnant, each pregnancy having terminated by miscarriage for reasons unknown.

It is of interest to note the observations of Murphy ('40) to the effect that abnormalities occur twice as frequently in whites as in negroes. In view of the statistical evidence that syphilis is much more common in negroes than in whites, the higher incidence of anomalies among whites tends to support the theory of a germinal origin, rather than a syphilitic origin, for congenital defects. Perhaps we may not thus easily exonerate the gonococcus but we still lack the positive evidence for its incrimination.

The obvious presence of amniotic adhesions in our case promptly raises the question as to their etiological significance. Some authors, including Murphy, still imply that such structures may be responsible for the development of fetal deformities but this longstanding belief now appears to be losing ground. Sir Arthur Keith ('40) emphatically declares that, "They are the result, not the cause, of fetal malformations." Keith's statement is based not alone upon his own observations but upon those of Bagg ('29), Streeter ('30), Ingalls ('32), and others. Similar adhesions seen in connection with fetal amputations are frequently not amniotic at all according to Streeter. Thus we see that amniotic adhesions, cords, and bands are seldom, if ever, factors in the production of anomalies. It is apparent that such structures, amniotic or not, may and do occur as dysplastic bands or adhesions secondary to fetal malformations (see attachment of amniotic surface of

placenta to fetal head, figs. 1 and 2). In the present case, the bands of amnion were definitely adherent to the head and face of the fetus. Keith believes that such structures are pathological formations which result from the growth and spread of fibroblasts in the deep fascia and cutis at the site of a "dysplastic lesion."

Excellent examples of congenital anomalies of endogenous origin have been experimentally produced by Stockard ('21), Bagg ('29) and others. Stockard employed such factors as chilling and oxygen deprivation on the ova of *Fundulus*. He arrested development almost to the point of a complete stop, using ova at their most critical formative period. The effects either of chilling or of etherized water on *Fundulus* eggs were essentially similar to those later produced by Bagg on the germplasm of mice by means of roentgen rays. In either instance a certain percentage of the surviving progeny were malformed in one way or another and to a greater or lesser degree. Bagg found that roentgen-rayed mice developed hereditary defects which were transmitted recessively according to Mendelian expectation. The results of such investigations as the foregoing lend additional support to the observational data favoring the theory of an endogenous origin for a majority of congenital anomalies.

The question naturally arises as to the causes of the initial germinal defects which are responsible for anomalies of development in man and animals under natural conditions. Such factors as prolonged exposure to cold, overdoses of either solar or roentgen radiation, and various types and degrees of febrile disease have been postulated by various authors. Perhaps any of the above plus various bloodborne agents such as pathogens and narcotics, if present in excess over long periods or at oft-repeated intervals may have such a deleterious effect upon the germplasm as to result in the production of germinal defects. Although such defects may or may not be hereditarily transmissible it is known that such human defects as harelip, cleft palate, and clubfoot may be transmitted according to the

laws of heredity, Kanavel ('32), Schroder ('35), Mau ('36), Fortuyn ('35), Ivy ('37) and others.

The origin of clubfoot has been satisfactorily explained by Bagg ('29). In this author's experiments with roentgen-rayed mice the malformed progeny of the irradiated parents revealed that very early in development a localized lymph stasis occurred as a bleb on either the dorsal or the plantar surface of the foot. These filled with extravasated blood which clotted and was generally resorbed shortly before or just after birth. This lesion, if severe, caused an arrest in development which in some cases produced clubfoot. Similar lesions were held responsible for anophthalmia.

An explanation of the origin of the cephalic anomalies found in our case may be derived from the writings of Streeter ('30) and Hertig and Rock ('41). These authors indicated the intimate relationship that exists between the early amnion and the germ disk in pre-villous human ova. Streeter stated that a defect in one or two cells at the junction of amnion and germ disk at this stage, especially if located in the future cephalic region, might result in malformations of the central nervous system including mouth, nose, and face. Lateral extension of such a defect might also include the amnion in the resulting monstrous development. If the defect involved a developmental arrest, portions of amnion would be continuous with the face or scalp, just as in a pre-villous ovum the amnion is continuous with ectodermal cells lying next to the primordium of the central nervous system.

In view of the foregoing it is evident that the severe cephalic anomalies described in this article must have resulted from a rather extensive defect in the anterior part of the germ disk occurring at about the stage of the early amniotic vesicle or sometime early in the first month and that the anomalous conditions were such as to result in the formation of amniotic adhesions between the craniofacial region and the placenta (figs. 1 and 2).

SUMMARY

A rare condition of complete vertical cleft of the face and mandible, including such unusual anomalies as bifid tongue, fully erupted tooth suspended by a pedicle, placenta bound to head by amniotic adhesions, bilateral anophthalmia and at least fourteen other malformations, is described. The incidence of the rarer anomalies is cited. Evidence favoring an endogenous origin for the majority of congenital deformities and discrediting the view that syphilis and gonorrhea are etiological factors in the causation of such anomalies is given. Keith's views to the effect that amniotic adhesions "are the result, not the cause, of fetal malformations" are reiterated. Possible causes of the initial germinal defects responsible for monster formation are briefly considered and include excessive exposures to cold, radiations, toxins, and fevers. It is admitted that extrinsic factors may, at times, be of etiological significance. The authors favor the explanation of Streeter in that craniofacial anomalies, particularly those associated with amniotic adhesions, may arise as a result of one or more defective cells at the point of junction between amnion and germ disk in a pre-villous ovum, probably during the second week of gestation.

ACKNOWLEDGMENTS

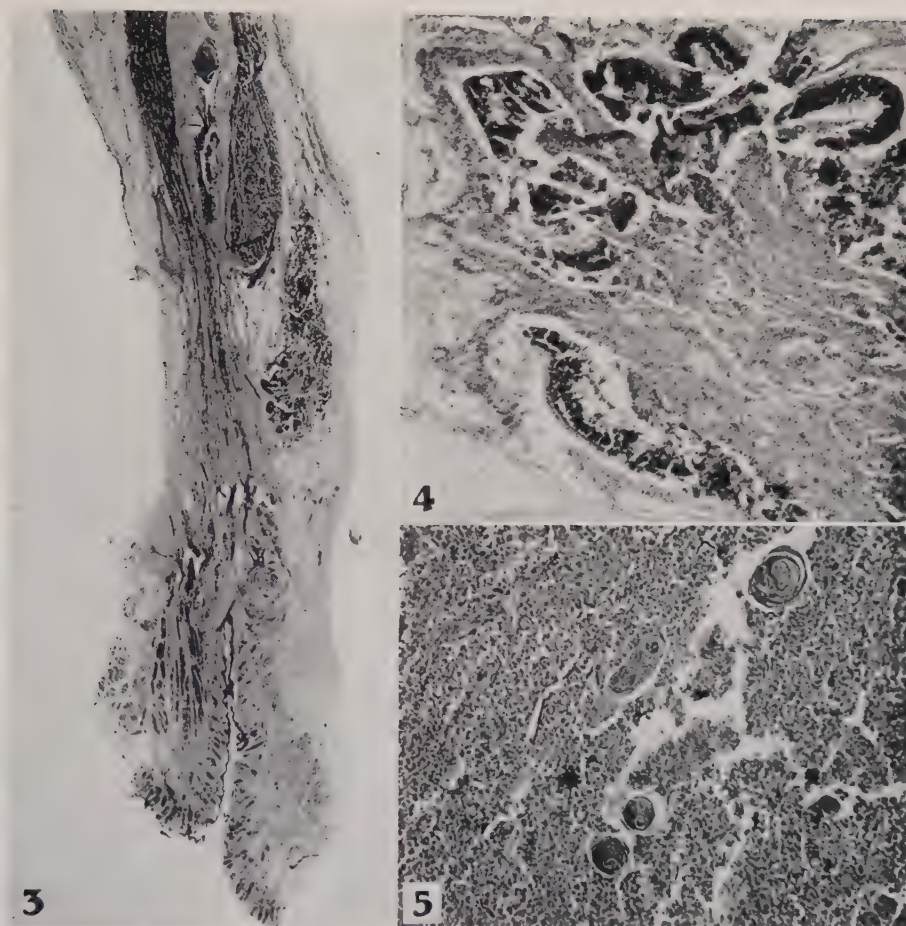
The authors wish to extend their warmest thanks to Dr. Lucy Lawrence, White Memorial Hospital, Los Angeles, California, for making the above reported case available for our study, and to Dr. Carrol S. Small, Department of Pathology, College of Medical Evangelists, for his assistance in the pathological interpretation of the tissues studied microscopically.

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3 Photomicrograph of section through region of right eye showing hypoplastic lids with ciliary hair follicles, below; extraocular skeletal muscles and cartilage, above; and irregular dark retinal fragments, surrounded by orbital fat, in the center. $\times 8.6$.

4 Higher magnification of retinal fragments seen in figure 3. $\times 54$.

5 Photomicrograph of a section through the pendant tag of thymus tissue which was attached to the left side of the monster's neck. $\times 162$. Note thymic corpuscles.

FAMILIAL VARIATIONS IN THE PATTERN OF RIB OSSIFICATION IN THE RABBIT¹

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ONE TEXT FIGURE AND ONE PLATE (ELEVEN FIGURES)

INTRODUCTION

Variation in the number of the several component parts of the axial skeleton is not common although many descriptions of such variation in a wide taxonomic range of animals have found their way into anatomical literature. Danforth ('30) was the first to suggest a possible hereditary background, and since then studies have been made of variations in man, swine, fowl and the rabbit (Green, '39). References to these papers may be found in that of Green. Although these authors agree that such variations are hereditary, in no case studied can the presence or absence of a skeletal unit be accounted for by a single gene substitution. Of the more complicated but entirely genetic explanations which have been offered, none has been adequately tested. Kühne hypothesized a single factor controlling a "cranial or caudal tendency in the vertebral column" implying a range of influence of the gene covering a more extensive region than a single vertebra. Backman ('34) suggested the possibility that such variations are the result of determining forces operating upon the growth and differentiation gradients of the body at the specific time when the structures are forming.

Although a single gene substitution is not adequate to explain the adult range of distribution of a character as it occurs in specific crosses, it is quite possible that a single gene could

¹ Part of a research program aided by a grant from the Rockefeller Foundation.

so affect growth of a particular region as to result in an approximation to Mendelian segregation; yet the interaction of growth in this region with that of neighboring regions might result in the sort of aberrant ratios so generally described.

Such an hypothesis is difficult to investigate satisfactorily until strains of animals of each contrasting type are found which can be depended upon to breed true. By selective breeding it has been possible in this laboratory to develop a homogeneous strain of twelve-ribbed rabbits and a strain of thirteen-ribbed rabbits which produces twelve-ribbed offspring so rarely as to make practicable the examination of the critical stages in embryonic development of the thoracolumbar region.

Generalized studies have been made of the ossification of the skeletons of man (Bardeen, '04 and Mall, '06), the rabbit (Minot and Taylor, '05), the rat (Strong, '25), the mouse (Johnson, '33) and the pig (Brockmann, '38) which have indicated the approximate times of rib ossification in these forms but have not considered the entire rib complex as a developing unit.

Rib formation is dependent in turn upon each of several processes: blastema formation, chondrification, calcification and ossification, which follow one another in that order. Since each of these processes requires a different sort of histological technique, the present study is concerned principally with the two distinct patterns of ossification which occur in twelve-ribbed and in thirteen-ribbed rabbits as found in the ribs individually and in the thoracic region generally.

MATERIALS AND METHODS

In the present study 127 embryos were secured at four developmental stages: 17 days 11 hours after coitus, 17 days 15 hours, 17 days 18 hours, and 18 days 11 hours. Sixty-two of these were twelve-ribbed embryos obtained from family X, a subline descended from Castle's small A race. In this family all of 148 progeny examined at birth or later have shown twelve fully developed pairs of ribs with no indication of variation in spite of the fact that the race is not greatly inbred,

and also that some adult individuals are known to have been heterozygous for the dwarf gene (Greene, '34).

The sixty-five thirteen-ribbed embryos were from extracted thirteen-ribbed mothers from a subline of the New Zealand White race known in our laboratory as family III. Of 353 offspring previously examined from this family 95.8% were thirteen-ribbed. The ten extracted females used gave 87.3% thirteen-ribbed animals out of 166 young produced prior to the operations for embryos. No one of the mothers of this race gave less than 70% thirteen-ribbed and most produced between 80 and 100%.

The embryos were removed through a lateral incision under as nearly aseptic conditions as possible and in several cases more than one set of embryos was obtained from a single mother. They were stained with alizarine red S, cleared in KOH and glycerine as described by Bruce ('41), and studied under a binocular dissecting microscope using a 7.5 X ocular and 40 mm. objectives. The ribs, humeri and femora were measured by means of an ocular micrometer calibrated in hundredths of a millimeter. Certain difficulties in measuring were encountered because the margin of ossification particularly in the ribs was not sharply defined; consequently, it was necessary to take measurements in the early stages from arbitrary points where the ossification appeared to be most marked. The smallest points of ossification, being less than .1 mm., were estimated at .05 mm.

OBSERVATIONS

The general process of ossification is similar in both families. At the earliest observed stage, 17 days 9 hours, the ribs of both races are hyaline cartilage, rendered transparent in glycerine thus distinguishing them from the surrounding tissue. As the rib begins to ossify, small diffuse areas about .05 mm. in length appear in the upper portion of the body of the rib just below the angle (figs. 2 and 10) ². In some cases these

² Although the areas of ossification are quite clear in all specimens under the microscope, small amounts and especially the limits of ossification in the early stages have been found very difficult to show photographically.

areas are larger indicating a more advanced stage, but the intensity with which they take the stain is the same throughout. The ossification centers of all of the ribs appear ventral to the angle and at about the same level in relation to the total length of the rib, but in actual measurements those of the central ribs, such as 5, 6 and 7 which ossify first, are more ventral. From each center ossification spreads rapidly and diffusely over a large area of the body of each rib in both a dorsal and a ventral direction, leaving irregular areas some of which take the stain more deeply. The lighter areas gradually fill in until the distribution of stain becomes entirely regular in the later stages. The description which follows of each of the four stages of each race is based upon the mean of ossification of each of the embryos taken at that stage. These averages are graphically represented in figure 1.

Embryos of the twelve-ribbed race

Stage 1. At 17 days 11 hours ossification was generally found on seven ribs, these being the third to ninth (figs. 2 and 10). Ossification was most advanced on the fifth, sixth and seventh and tapered off on the third and fourth and on the eighth and ninth. In one of the more advanced embryos the second pair of ribs had a small center.

Stage 2. At 17 days 15 hours, 4 hours later, ossification had usually extended to include eight ribs, being the third to the tenth (fig. 4). In a few embryos the first indications of ossification were apparent in the second, eleventh and twelfth. The amount present on the fourth to the ninth ribs in all the embryos was greatly increased over that of the first stage.

Stage 3. At 17 days 18 hours the number of ribs which had ossified was the same as in the previous stage, but the amount of bone on each rib had increased considerably both in the dorso-ventral direction and in thickness (fig. 6). There seemed to be a tendency for the ribs anterior to the fifth to ossify more rapidly than those posterior to it. However, the gradual taper in both directions remained.

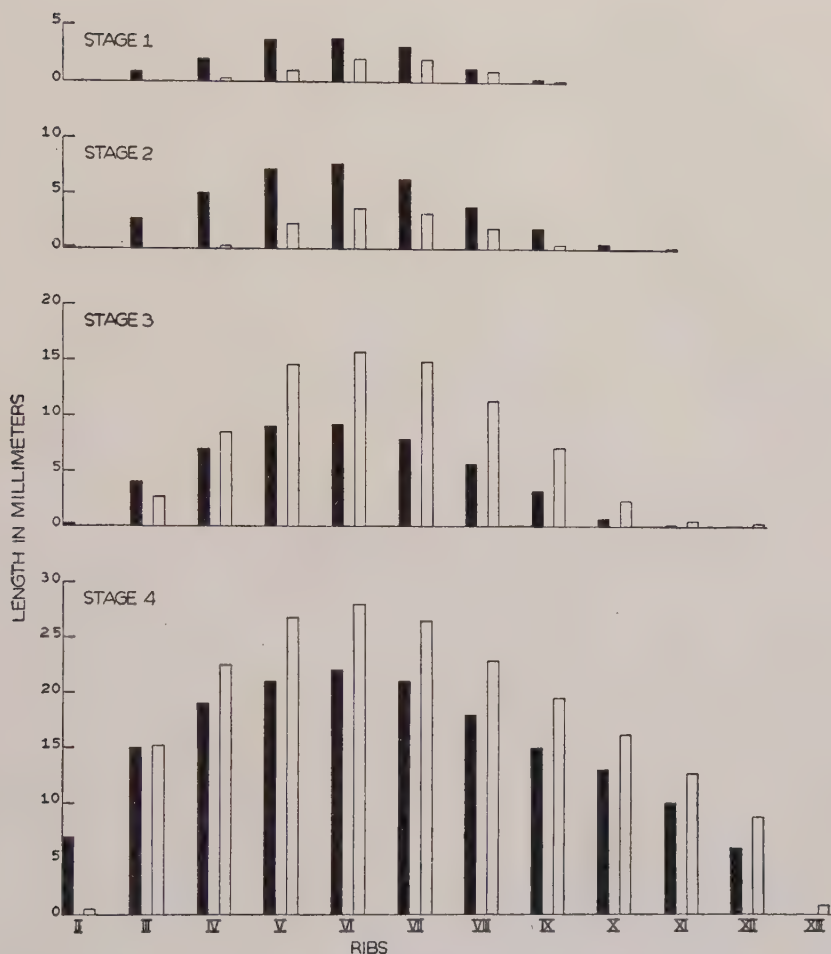


Fig. 1 The average length of ossification of ribs from embryos taken at four stages in development. The black columns represent the twelve-ribbed family and the white the thirteen-ribbed family. Stage 1, fifteen small race embryos and twenty-six large race embryos taken 17 days 11 hours after coitus; stage 2, twenty-two small race embryos and nine large race embryos taken 17 days 15 hours after coitus; stage 3, twenty small race embryos and ten large race embryos taken 17 days 18 hours after coitus; stage 4, five small race embryos and twenty large race embryos taken 18 days 11 hours after coitus.

Stage 4. At 18 days 11 hours ossification had increased so as to include the second and the twelfth ribs which apparently began to ossify at about the same time. As in the last stage, the ribs at the anterior portion of the basket tended to be slightly more advanced with the exception of the first and second which were markedly retarded (fig. 8). The second was very much shorter, and the first had not begun to ossify at all and was therefore the last to show ossification. In no case was it found to have passed beyond the calcified cartilage stage as indicated by its granular appearance.

One litter composed of five animals was obtained at this stage, four of which possessed approximately the same amounts of bone in the rib region and one of which possessed no bone whatsoever. Consequently, the normal average amount of ossification on each of the ribs is probably somewhat larger than the average here recorded.

Embryos of the thirteen-ribbed race

Stage 1. At 17 days 11 hours ossification was found in most cases on four ribs including the fifth to the eighth of which the sixth and seventh showed the greatest amount. Figures 3 and 11 show an embryo slightly more advanced than the average. A few showed a small center in the fourth and ninth, but in fourteen embryos none of the ribs showed any ossification at all. This is reflected in the average amount of ossification portrayed in figure 1.

Stage 2. At 17 days 15 hours in only one case had ossification extended to ribs other than the fourth to the ninth. This was in the tenth rib on one side only. The amount of ossification on each rib, however, had increased materially as indicated in figure 1. The ossification of the embryo shown in figures 5 and 12 is somewhat less than average.

Stage 3. At 17 days 18 hours the average number of ribs which had ossified increased to ten including the third to the twelfth. The fifth, sixth and seventh had progressed about equally and the third, fourth, eighth, ninth and tenth tapered off at either end. In some cases as in figure 7 the taper was

rather abrupt. Only three embryos showed ossification on the second. The entire group showed a tremendous increase in ossification over that of the embryos of the previous stage.

Stage 4. (18 days 11 hours) During the succeeding 17 hours ossification progressed so that in most embryos all the ribs except the first, second and thirteenth were partially ossified (fig. 9). The first had not ossified in any embryo. Only four embryos out of twenty contained small areas on the second, and six had some ossification on the thirteenth. The twelfth in many cases showed evidence of ossification sooner than the second. At this stage ossification had reached the head of the rib dorsally and had extended toward its definitive end ventrally.

TABLE 1

The average lengths of ossification of the humerus and femur in sixty-five embryos of a family of large body size, and sixty-two embryos of a family of small body size, taken at four developmental stages.

RIB TYPE	AGE		LENGTH OF HUMERUS IN MILLIMETER		LENGTH OF FEMUR IN MILLIMETER	
	Days	Hours	Left	Right	Left	Right
12	17	11	.380	.393	.224	.238
	17	15	.483	.493	.290	.290
	17	18	.516	.510	.338	.341
	18	11	.830	.830	.572	.572
13	17	11	.237	.215	.127	.127
	17	15	.311	.274	.124	.124
	17	18	.571	.571	.360	.360
	18	11	.940	.908	.658	.654

From these observations it is apparent that in both strains ossification begins on the central ribs and progresses in both dorsal and ventral directions on each rib and in anterior and posterior directions throughout the rib complement.

It is evident from the first stages of the two races that the ribs of the small race rabbits begin to ossify earlier than those of the thirteen-ribbed strain, and it is evident from the later stages that ossification tends to be centered more posteriorly in the large race than in the small. One of the most obvious

differences is the great rapidity of ossification in the thirteen-ribbed group during the 3-hour period between the second and third stages. This is made more conspicuous since the increase is especially pronounced in the posterior ribs.

The question arises as to whether these differences observed in the ossification of the ribs of these two families are specific for that region or whether they are characteristic of the ossification process occurring throughout the animal. In an attempt to decide between the two alternatives, the humerus and femur of the same embryos were measured. The data from table 1 show in the thirteen-ribbed family the same delay in ossification accompanied by a similar sudden spurt between the second and third stages, and the same final increased amount of ossification.

Asymmetry

A certain amount of asymmetry in the expression of extra ribs has been quite generally recognized in all studies of rib variation. Observations of the asymmetries manifest in early stages are therefore important in determining the manner in which they, as well as the character itself, arise.

In describing the asymmetry of rib ossification it can be considered in several ways: (1) the total amount of ossification on each side; (2) the relative position of ossification centers with respect to the mid-dorsal or mid-ventral line; (3) the anterior or posterior ossification range of the two sides; and (4) the asymmetries of the individual ribs as compared with the general rib ossification pattern.

As shown in columns 8 and 9 of table 2, there is a tendency for the total amount of ossification to be greater on the right side in the twelve-ribbed family and on the left in the embryos from the thirteen-ribbed group. Although the incidence of such asymmetry is not great, it indicates that the forces determining the ossification pattern of the two sides are not exactly the same.

TABLE 2
A comparison of the total amount of rib ossification and its asymmetrical distribution with respect to its anterior-posterior position and the amount on the left and right sides in the 127 embryos of the two families (see text).

RIBS	AGE		LIT-TERS	EM-BRYOS	TOTAL OSSIFICATION					INDEX OF POSITION				
	Days	Hours			No. greater on left	No. equal	No. greater on right	Mean		No. less than 100	No. equal to 100	No. greater than 100	Mean	Range
								Left	Right					
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	
12	17	11	3	15	2	6	7	.99	1.27	9	4	2	92.45	70.54-156.21
	17	15	3	22	9	4	9	3.45	3.63	11	2	9	93.45	57.36-157.89
	17	18	4	20	9	0	11	4.68	4.71	10	1	9	96.27	67.57-136.36
	18	11	1	5	2	1	2	16.78	16.70	3	1	1	99.82	97.21-102.94
13	17	11	3	26	5	15	6	.64	.49	4	16	6	103.64	33.33-253.79
	17	15	3	20	4	12	4	.65	.59	4	12	4	103.00	57.04-181.25
	17	18	2	9	7	0	2	8.50	8.22	5	0	4	99.98	93.72-112.38
	18	11	2	20	13	0	7	20.08	19.94	10	0	10	100.53	87.92-110.54

With respect to the dorso-ventral position, ossification seems to progress equally in both directions.

In order to study the symmetry, particularly of the anterior-posterior range, the relative position of rib ossification was evaluated by multiplying the amount of ossification of each rib by its numerical position. A ratio of the mean of all such (twelve or thirteen) products of one side to that of the other provides an index which is 100% in case of perfect symmetry, and the departures from which supply a measure of asymmetry—arbitrarily the left was the numerator. Thus, percentages less than 100 represent greater ossification in the anterior region of the left side; whereas, percentages greater than 100 represent greater ossification in the anterior region of the right side.

The comparison of the embryos on the basis of both amount and range of ossification on the two sides shows that the twelve-ribbed group has greater ossification in the anterior region on the left; whereas, in the thirteen-ribbed family it is on the right as shown by both the average indices (table 2, column 13) and the range of indices (column 14). In general the sides with the greater amount of ossification have it in the posterior region. It should also be noted that with regard to position the twelve-ribbed family is characterized by a high proportion of asymmetrical individuals, only eight of the sixty-two individuals being symmetrical; whereas, in the thirteen-ribbed family there is more of a tendency for asymmetry in the early stages but not in the later.

Occasionally during the early stages one of the marginal ribs in a group of three or four is slightly more precocious than the others, but during most of the process ossification progresses from one rib to the next evenly. It may vary from side to side in an individual or from family to family, but the entire set of ribs on one side of an embryo of each family appears to ossify as a unit.

In the long bones the slight but not significant asymmetry which is shown in table 1 is similar to that found in the ribs.

DISCUSSION

Ossification is a precise process which begins at a definite time following the completion of preliminary calcification. Johnson ('33) in a study of ossification of the skeleton of mice refers to the tendency for an almost continuous development of the skeleton from the blastema through ossification with small intervals of time occurring between completion of chondrification and initiation of calcification and between calcification and ossification. While this is also true in the rabbit, it is apparent from the present observations that there are marked differences in the pattern of ossification in the two families described. Further evidence of the existence of such pattern differences among rabbits, at least with respect to the time of onset of ossification, is to be noted in comparing these observations with those of Minot and Taylor ('05). In their rabbits ossification of the ribs occurred approximately a day later than that in either of the present races. Since the entire process of rib ossification is completed in about 30 hours, the difference is highly significant. Although Minot employed routine histological methods including sectioning for detection of ossification centers, it has been shown by Mall ('06) that smaller ossification centers can be identified in alizarin stained whole mounts. Whereas some of the discrepancy can be explained through the difference in techniques employed, it seems more probable that in view of the differences noted in the present families that Minot's rabbits were inherently different.

Since there is a difference in adult body size between the two families, the ossification difference may be one associated with size rather than being specific to twelve- and thirteen-ribbed embryos generally. The fact that none of the fourteen twelve-ribbed embryos observed in family III show an ossification pattern outside the range of variation manifest by the thirteen-ribbed members of that group seems to be evidence for a familial association of this kind.

From studies of body weight and bone measurements in small and large sized races of rabbits and the subsequent generations derived from a cross between them, Castle ('14 and '22) concluded that the observed differences were due to the general growth rate. This view found additional support when the early embryonic stages were shown by Castle and Gregory ('29) to exhibit differences in the rate of cell division. This difference in rate together with a prolongation of growth at maturity easily accounts for the marked differences in adult body size and might also account for differences in ossification pattern. However, Wright ('32) by special statistical analysis applied to Castle's data was able to show evidence of differences in growth forces specific to certain regions. Other regionally specific effects have been observed externally associated with such genes as short ear in the mouse (Green and Green, '42) and in the Creeper fowl (Landauer, '39).

Based on these observations two alternative explanations for these differences in ossification may be suggested. First, each of the observed differences, time of onset, rate and regional growth, may be due respectively to separate genes. The marked acceleration between the third and fourth stages of the thirteen-ribbed family in itself could be explained in this way. In connection with such a view it is also important to note that the number of ribs in the twelve-ribbed individuals occurring in the thirteen-ribbed race appears already to have been determined before ossification began. Thus the actual number of ribs is determined at or prior to chondrification.

The second alternative is that these differences are determined by the interrelation of growth and differentiation. Inasmuch as the difference in the gestation period is a matter of only a few hours in these two families, and that the young, so far as can be seen, are in the same stage of maturity at parturition, it is possible that the same temporal relationship holds for other fundamental processes. The thirteen-ribbed race being the larger, it may be assumed from the results of Castle and Gregory that cell division and therefore growth is proceeding more rapidly. Thus, at the time ossification normally

begins the larger volume of the embryo as compared with the area of ossification being initiated may act in such a manner as temporarily to retard the actual visible process. However, once ossification is begun, it would naturally tend to proceed more rapidly than in the smaller race due to the generally faster growth or metabolic rate; thus accounting for the spurt in the later stage of ossification. Little is known about the conditions inducing the physiological precipitation of calcium leading to ossification; therefore, the difference in the point of origin of ossification in the two races might be merely a manifestation of delay in initiation of the general process.

The strongest evidence for the latter hypothesis is to be found in the fact that acceleration of ossification in the thirteen-ribbed family occurs simultaneously in ribs and limbs.

The variations in symmetry in the ossification pattern also seem to be more in harmony with differences in the general growth rate between the two sides than to local differences of ossification as indicated by the specific tendencies in the two families since both body size and asymmetry appear to be inherent and correlated.

Although asymmetry in the long bones is slight as compared with the ribs, the tendencies are in the same direction and might readily be explained by the difference in form of ossification. In the former it progresses as a compact cylinder; whereas, the periosteum of the ribs is diffuse extending for some distance along the margins before the central areas are ossified.

The present material does not permit discrimination between the two interpretations suggested, but the existence of such internal differences in growth pattern in homegeneous stocks of laboratory animals as have here been demonstrated provides an entirely new approach to the analysis of mammalian development, differentiation and conformation.

SUMMARY

Embryos from a twelve-ribbed family (X) of rabbits and a thirteen-ribbed family (III) taken at four developmental

stages exhibited familial patterns of rib ossification differing in time and position of onset and speed of progression.

Ossification began on the central ribs, progressed in a dorso-ventral direction on each rib and in an antero-posterior direction to complete the rib complement. In the twelve-ribbed group ossification began earlier, originated more anteriorly and progressed more rapidly on the anterior ribs than that in the thirteen-ribbed family. In the latter group differentiation was in general greater and was characterized by a sudden acceleration between the second and third stages.

Asymmetry in the total amount of ossification on the two sides and in the rate of progression anteriorly and posteriorly was noted in both families although the ossification on the individual ribs in a dorsal-ventral direction seemed to be symmetrical.

Alternative explanations are suggested for separate familial patterns, one based on regional growth forces and the other on the interrelation of growth and differentiation.

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PLATE 1

EXPLANATION OF FIGURES

Representative embryos from each of the four developmental stages described in the text. The embryos to the left, even numbers, are from the twelve-ribbed family; those to the right, odd numbers, are from the thirteen-ribbed family. The last three figures are enlargements of the region of rib ossification of the youngest embryos.

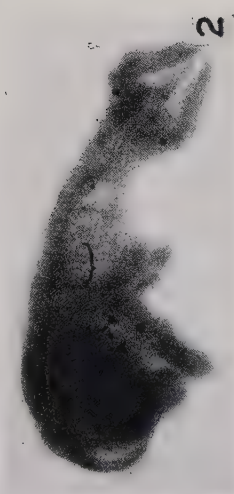
2 and 3 17 days 11 hours. Note greater ossification in figure 2.

4 and 5 17 days 15 hours. Compare the amount of ossification in the two embryos at this stage with those of the next.

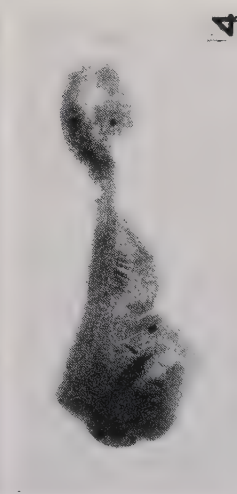
6 and 7 17 days 18 hours. The ossification in the thirteen-ribbed embryo is slightly although not noticeably greater.

8 and 9 18 days 11 hours. Note the ossification on eleven ribs in figure 8 and twelve ribs in figure 9. Ossification is just beginning on the second and third ribs in the thirteen-ribbed embryo. The posterior ribs are well advanced.

10, 11 and 12 Enlargements of figures 2, 3 and 5 respectively.



2



4



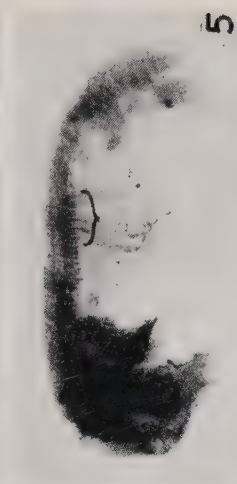
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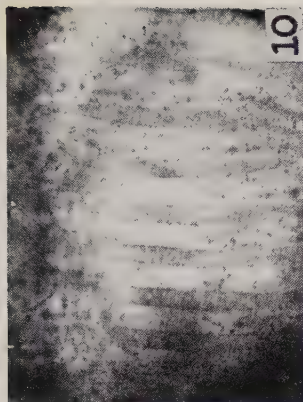
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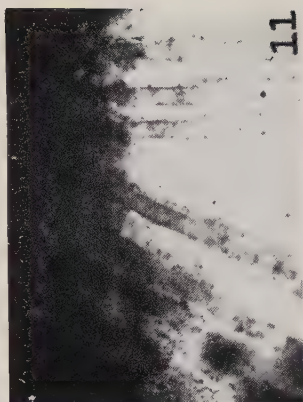
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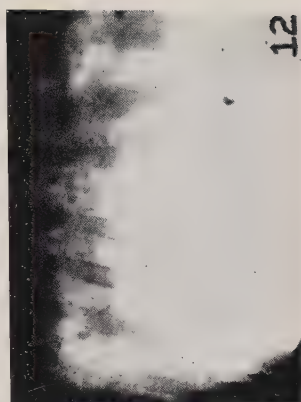
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11



12

ENDONEURIAL EDEMA IN CONSTRICTED NERVE

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THIRTEEN FIGURES

This is a report of certain observations on intraneural edemas, made in the course of nerve splicing experiments, which indicate that seepage of fluid goes on continually in the endoneurial spaces. At the same time, these observations point the way to a practical method of collecting the intraneural fluid in amounts large enough for biochemical analysis. Inasmuch as this fluid constitutes the medium in which the nerve fibers normally live and in which, as we shall discuss below, they seem to grow best during regeneration, information about its source, composition and behavior is desirable for both theoretical and practical reasons.

The observations reported here were made in nerves which had been severed and reunited by means of segments of artery, the so-called sleeve-splicing method described previously (Weiss, '41 b, '43). In such cases the proximal nerve stump reacts differently depending on the size of the arterial sleeve. If the arterial lumen is as wide as the nerve in its interior, the proximal stump will show no more than the usual traumatic reaction of a transected nerve, which means ascending degeneration for a few millimeters from the cut, followed by the regenerative outgrowth of new axons from the level at which this process is arrested. If, however, the artery is of smaller

¹ This work was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Chicago. It was also aided by the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago. Assistance in the operations by Dr. A. Cecil Taylor is gratefully acknowledged.

diameter, so that it had to be forced over the nerve end by distension, the subsequent constriction of the sleeve produces local compression of the nerve of various degrees, and this, in turn, affects the condition of the proximal stump much more profoundly than does mere transection. Even arteries which are apparently sufficiently wide at rest to receive the nerve in their interior may later produce constriction owing to the gradual contraction of their muscular wall. This contracture becomes particularly evident under the influence of adrenalin. Adrenalin applied to an arterial sleeve produces heavy constriction of the latter followed by block of impulse conduction, and frequently morphological degeneration, of the compressed nerve fibers (Weiss and Davis, '43). Arteries of only slightly insufficient width produce effects of much less severity. In all cases of constriction, however, whether moderate or extreme, a fairly large amount of fluid was found to accumulate in the endoneural spaces at the central side of the bottleneck. It is on this endoneural edema that we shall focus our attention in the present report. Another associated phenomenon, the damming up of axonal substance proximal to the constriction, will be dealt with in a later paper.

MATERIALS AND METHOD

Edemas following constriction or other obstruction of the nerve have thus far been observed in sixteen chicken and fourteen rat nerves between 1 and 17 weeks after the operation. Five chickens had edematous records on nerves of both wings, while the rats were always operated on unilaterally. The chickens were operated on at an age of between 5 and 7 months; the rats at an age of between 2 and 10 months. As the operation was described in detail in an earlier publication (Weiss, '43), only the main steps may be repeated here. The segment of artery to be used as splicing sleeve is slipped over a specially designed forceps-like instrument. The cut end of the proximal stump of the nerve is then grasped between the two prongs of this instrument and the artery is pulled from the instrument over the seized nerve end. The cut end of the distal stump is

then introduced into the open end of the artery by means of a so-called spreader. For our present purpose, it is important to remember that when it is stripped from the splicing forceps, the artery is sufficiently distended to slide easily over the nerve stump; likewise, the cylindrical shape of the splicing instrument precludes crushing of the nerve during manipulation. Therefore, none of the effects observed in the preparations can be ascribed to damage sustained in the operation. This is further confirmed by the fact that only those nerve splices showed edematous involvement of the proximal stump in which the artery had subsequently contracted, while no effects were noted in cases in which the nerve had remained uncompressed, although both types had been manipulated in exactly the same way.

In some cases, the central and peripheral stumps of the severed nerve were simply united; while in other cases, a fragment of the peripheral nerve was spliced to the proximal stump as a graft. In the latter cases the graft ended freely with an open end. Of the plain nerve reunions some were done with close apposition of the nerve ends while in other cases, a gap was left between the nerve ends in the sleeve. This gap was either left open or filled with blood plasma. The latter procedure stimulated the formation of fibrous tissue in the gap.

All histological studies were made after fixation in Bouin's fluid and silver impregnation according to Bodian, frequently supplemented with a Mallory triple Azan stain. Measurements of nerve cross sections were made by planimetry of camera lucida drawings, while the diameters of longitudinally sectioned nerves were determined by measuring the greatest width appearing in the serial sections.

PROXIMAL EDEMAS

Gross inspection of nerves spliced with a tightly fitting arterial sleeve reveals swellings of the nerve both at the proximal and at the distal end of the sleeve. Of these, the proximal one is much more conspicuous while the distal one may be

completely absent. As we shall demonstrate presently, the two swellings are of different nature, only the proximal one being caused by edema. Figure 1 reproduces a nerve which has already been described on an earlier occasion in connection with studies on the physiological effects of nerve compression (Weiss and Davis, '43). It may serve here as an introductory example, though it does not represent a severed and reunited nerve, but a specimen in which the sleeve of artery was simply pulled over a proximal nerve stump bracelet-fashion. In the

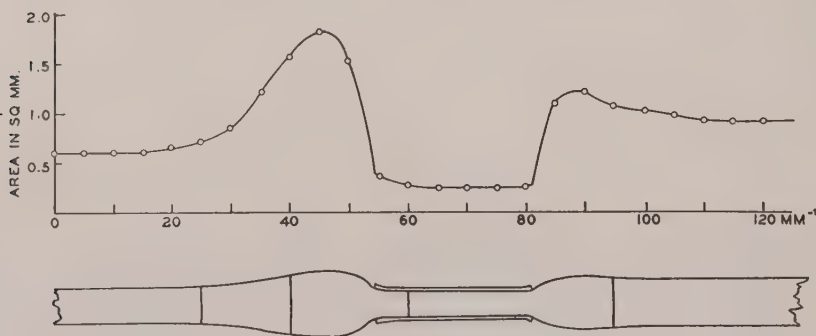


Fig. 1 Edematous tibial nerve (rat R50) constricted by undersized arterial sleeve, 10 days after operation. Lower diagram: Profile of nerve in correct proportions. Upper graph: Cross-sectional area at 0.5 mm. intervals, plotted over length of nerve. Proximo-distal direction from left to right.

lower part of the figure the nerve is reproduced in true proportions, as reconstructed from measurements of cross sections. The proximo-distal direction is from left to right. The upper part of the figure gives the values of cross sectional areas measured at intervals of 0.5 mm. each, plotted over the length of the nerve. One notices that the diameter of the nerve begins to swell more than 3 mm. proximal to the sleeve, first gently, then rather steeply, reaching a maximum of about three times its original size 1 mm. proximal to the sleeve. It then declines abruptly to the narrow dimensions of the bottleneck. At the emergence from the sleeve, at mark 80, the diameter increases again with a slight hump. The fact that the whole distal portion of the nerve is larger than the

proximal part to the left is due to Wallerian degeneration. The compression has produced degeneration of most of the fibers distal to the constricted area, and since freshly degenerating nerve fibers are swollen, the distal nerve as a whole has thickened. The proximal swelling, on the other hand, is entirely due to the accumulation of interstitial fluid between the nerve fibers (fig. 4 in Weiss and Davis, '43).

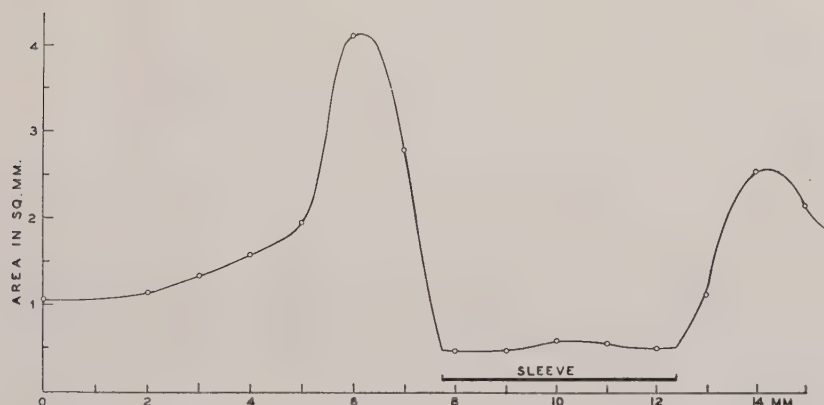


Fig 2 Variation of cross-sectional area of edematous sciatic nerve (rat R7; 200 g.) severed and spliced with sleeve of carotid artery (donor: 360 g.), 5 weeks after operation. Oscillographic tests of this case have demonstrated conductivity of regenerated fibers through the sleeve. Proximo-distal direction from left to right.

While this nerve was fixed only 10 days after the operation, specimens preserved after much longer intervals show essentially the same situation. In the graph, figure 2, cross sectional area of a sleeve-spliced nerve 5 weeks after the operation is plotted over the length of the nerve. The nerve within the sleeve has been compressed to about one-half of its normal size at more proximal levels. Proximal to the sleeve, there is a bulbous edema, the beginnings of which can be traced about 7 mm. upwards in the nerve and which, at its maximum width just in front of the sleeve, distends the nerve to about four times its original size. These two examples are representative for the rest.

Figure 3 represents in summary fashion the measurements obtained in all sixteen chicken and fourteen rat specimens. Diameters were measured at three levels as indicated: at a far proximal level of the nerve (P); at the level of the edema at its maximum width (E); and at the level where the sleeve is narrowest (S). The graph reveals several facts. Firstly, it

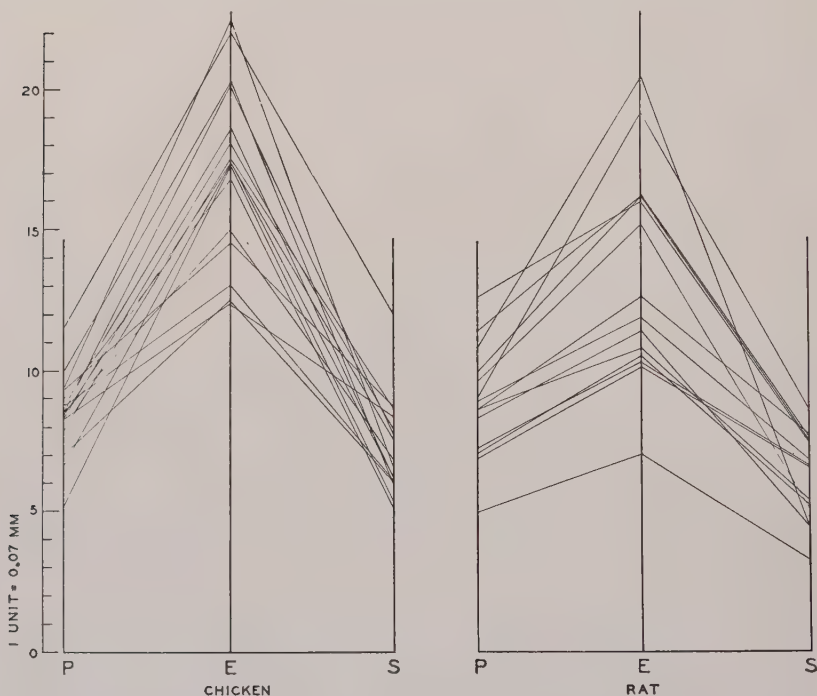


Fig. 3 Size of edemas of thirty nerves with constrictions. Ordinates: Nerve diameter. P, proximal normal part of nerve; E, edema at greatest width; S, sleeve at narrowest point.

can be seen that the chicken, by and large, shows greater accumulation of edematous fluid than the rat. Secondly, in the rat, the diameter of the nerve inside of the sleeve (S) is usually well below that of the proximal stump (P) (figs. 1 and 2), while in the chicken, the difference is much less pronounced although the edema, nevertheless, is well marked. In some cases, the diameter of the nerve in the sleeve is even larger

than that of the proximal nerve, but this is due to a secondary widening of the sleeve long after the proximal edema has become established. Thirdly, while there seem to be two distinct classes, one with mild edema and one with considerable edema, there is no proportionality between size of the edema and size of the nerve. The difference between the diameters at the level of the swelling (E) and in the unaffected portion of the nerves (at P) is approximately the same in small and large nerves. This would mean that the amount of accumulated liquid is relatively independent of the size of the nerve. Nevertheless, in order to give a general idea of the order of magnitude of the swelling, we have calculated the average ratio of cross sectional area at the widest point of the edema over cross sectional area of the unaffected proximal nerve. The values are 4 and 2.3 for the chicken and the rat, respectively.

Once it has appeared, the edema does not seem to change appreciably in size. This conclusion has been reached from a study of the size of the edema in relation to its age. Figure 4 shows a record of all thirty observed cases in which the size of the edema was plotted against the post-operative age of the specimen. White circles represent rat, and black circles chicken cases. The largest diameter of each edema was chosen as representative of its size. Ratios of edema diameter over proximal nerve diameter were plotted as ordinates. If we consider the rat nerves first, it can be seen that animals studied as early as 1 week, or as late as 17 weeks, after the operation show edemas of approximately the same extent, with the cases preserved at intermediate times falling in line. The chicken cases are distributed over a shorter period, but likewise cluster around a nearly steady average value. The five pairs of chicken data connected by bars represent values obtained from nerves of opposite wings of the same individual. In these cases, the nerves of both sides had been severed and reunited with certain asymmetries of procedure; for instance, apposition of nerve ends on one side, intra-arterial gap on the other; or empty gap on one side, gap filled with a blood clot on the other; etc. Even so it can be seen that paired values for op-

posite nerves of the same individual lie within a close range. Whatever asymmetries were observed between the two sides can presumably be attributed to the asymmetrical condition of the operated nerves (see below).

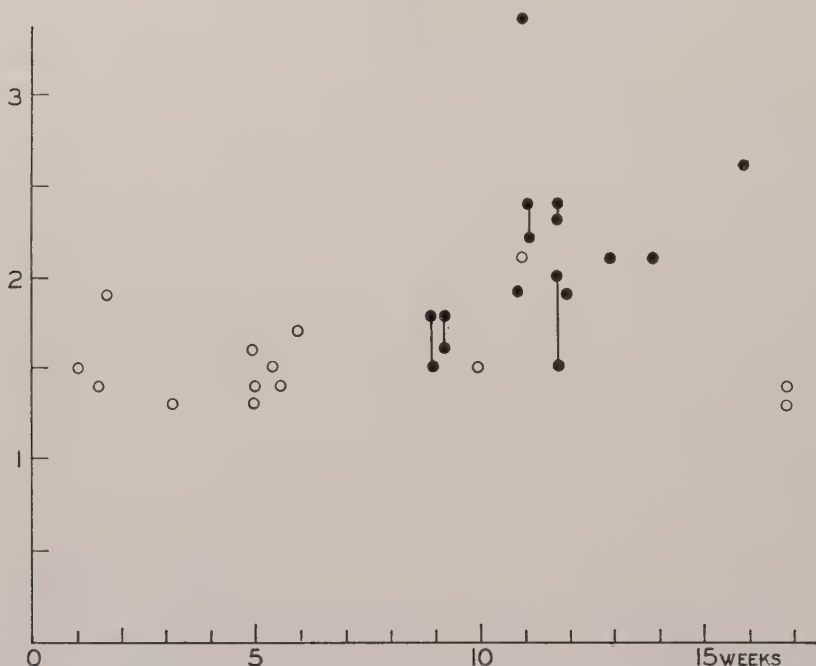


Fig. 4 Distribution of edemas in respect to postoperative age. Ordinates: Ratios of diameter of edema over diameter of proximal nerve ($\frac{E}{P}$ from fig. 3). ○ rat; ● chick. Vertical bars connect paired experiments on symmetrical nerves of same animals.

We may conclude, therefore, that the edema is established in its full size within a week of the appearance of the constriction, and the bulbous shape of the nerve produced by the edema will be maintained without appreciable change, either increase or diminution, for at least the 4-month period over which our observations extend.

The observations thus far reported may be summarized as follows. Constriction of a nerve by an arterial sleeve, used either as a jacket over an intact portion of a nerve or as a

splice over reunited ends of nerve stumps, causes the accumulation of edematous fluid in that part of the nerve trunk which lies immediately proximal to the constriction. The liquid always accumulates at the near side of the sleeve and reaches its maximum extent immediately prior to the entrance of the nerve into the bottleneck. The swelling tapers off in the proximal direction. Once established, it remains in evidence for several months. The location, distribution and size of these edemas point to the existence in the endoneurial spaces of a steady centrifugal seepage of fluid which, when peripherally obstructed by nerve compression, becomes dammed up and distends the nerve locally. A further discussion of this will be presented below.

HISTOLOGICAL OBSERVATIONS

Cross sections through the normal and edematous regions of a nerve during the early stages of constriction have been presented in a preceding paper (Weiss and Davis, '43). A later stage is illustrated here in figure 5. The picture shows cross sections through the unaffected proximal part (A), the edematous region (B), and the constricted sleeve region (C) of a sciatic nerve spliced 5½ weeks previously. The swollen region of this nerve is distinguished from the normal region by the wide open clefts which have appeared between the various nerve bundles, and the dissociation of the nerve fibers within each bundle. Since normal rat nerve trunks show no very distinct fasciculation, we have no way of telling whether or not the cleavage planes along which the edematous nerve breaks up into distinct fascicles correspond to the peripheral grouping of the fibers. Even between the individual fibers, however, the endoneurial meshes have become greatly distended, leading to wide separation of the nerve fibers from one another.

Of special interest is the fact that the edematous substance filling the widened spaces shows up in the fixed preparations as a precipitate impregnated with silver. In figure 5B, this granular mass is particularly well defined in the larger spaces.

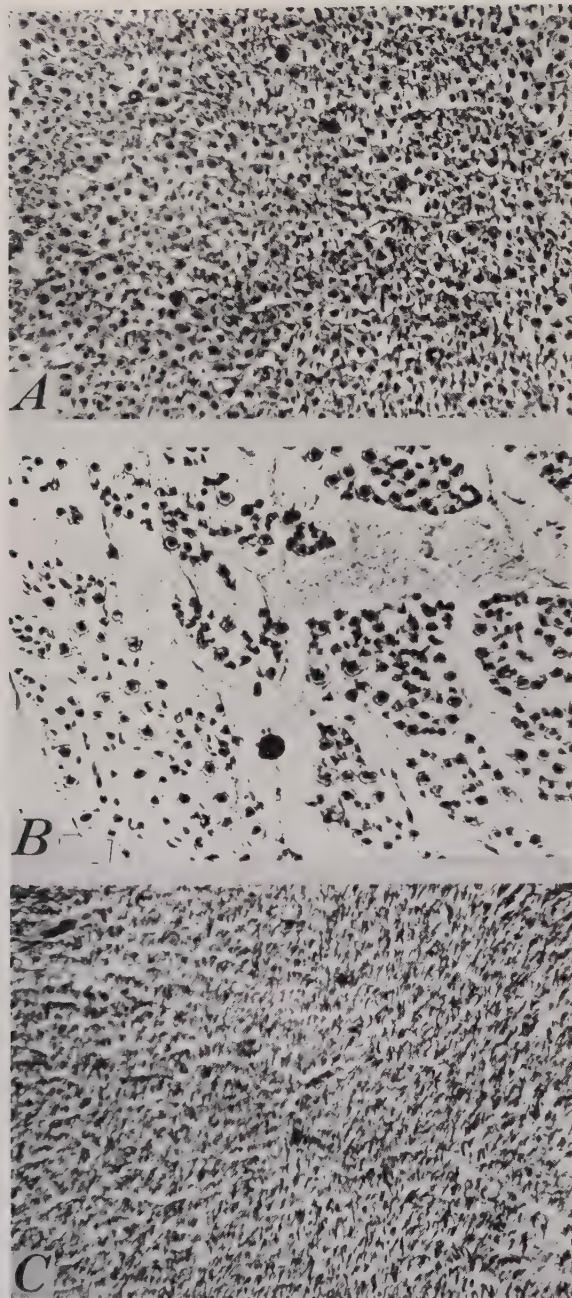


Fig. 5 Packing of nerve fibers in three different regions of constricted edematous rat nerve, sleeve-spliced 5½ weeks previously. x 250. A, proximal unaffected part; B, edematous region; C, sleeve (fiber course slightly oblique).

Under high magnification, it resolves itself into a very fine reticulum, of the sort familiar to the histologist in precipitates of fiber proteins treated with silver. Therefore, judging from the histological evidence, the endoneurial edematous fluid contains coagulable protein.

Figure 5C, a section through the compressed segment distal to the edema, shows that in this region the endoneurial spaces are largely obliterated. Whatever small spaces may have



Fig. 6 Nerve fibers in edematous bulb of sleeve-spliced chicken nerve C13R, 66 days after the operation. x 230.

persisted, are obviously inadequate for the complete drainage of the vast amount of liquid present in the edematous region.

Figure 6 is from a longitudinal section through the edematous region of a constricted chicken nerve (radial), 2 months after the operation, and illustrates the separation among individual nerve fibers or small nerve fiber groups produced by the imbibition of the endoneurial tissue with the edematous fluid.

While during the first week after its appearance, the edema does not seem to contain a significantly larger number of cells than could be expected to have been preexisting in the endoneurium, later on the cell content in the liquid-filled meshes gradually increases. Most of the new nuclei are round to ovoid, with the smooth contour of fibrocytes, and are settled along fine fibers which begin to span the originally liquid

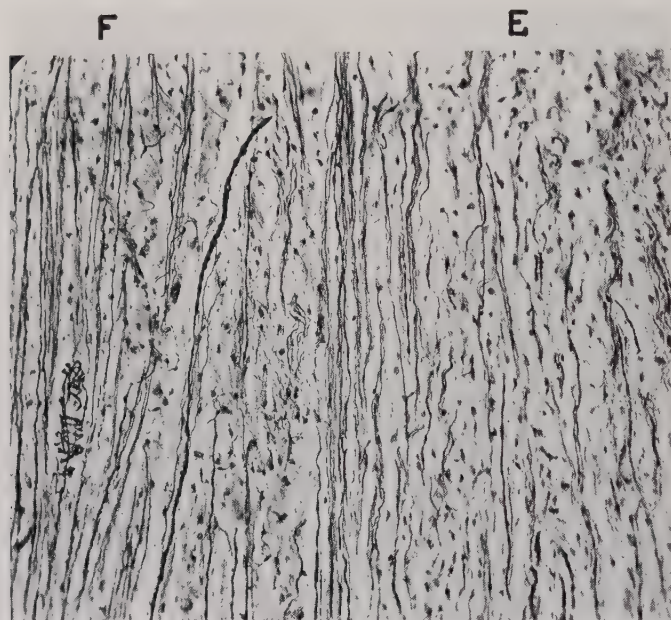


Fig. 7 Edematous region of sleeve-spliced chicken nerve C12L, 97 days after operation. $\times 120$. The picture shows transition from edematous condition (E) in the interior to fibrotic condition (F) near the surface of the bulbous swelling. Note spiral apparatus of Perroncito formed by an occasional regenerating fiber encountering growth resistance in the fibrotic area.

spaces. Within a few months, this spongy tissue becomes more consolidated, primarily in the peripheral portions of the bulb (fig. 7). The nuclei assume more irregular outlines, some look atrophic and some show signs of pyknosis. As the intercellular matrix becomes denser, the cell population in it seems to decline. The formation of this first loose and later

dense connective tissue begins at the most distal pole of the swelling, i.e., immediately in front of the entrance to the sleeve. From here it may gradually extend proximad, but even so, it always remains confined to the periphery of the bulb, while in the center wide liquid spaces persist. The fact that the central portion of the edema can resist fibrous transformation for several months suggests that the edematous fluid contains an antifibrotic principle, which partly explains its superior properties as a medium for nerve regeneration (see below).

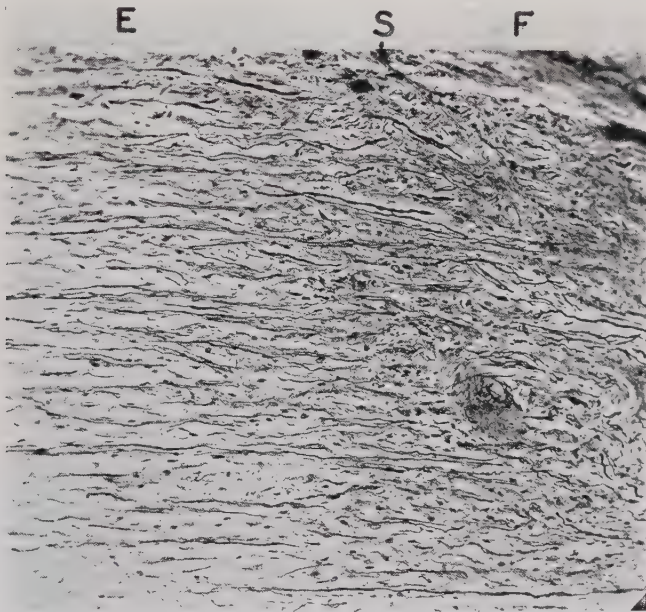


Fig. 8 Transition from proximal edematous (E) to distal fibrotic (F) zone of sleeve-spliced nerve (chicken C18R), 90 days after operation. $\times 120$. Entrance to sleeve at S. See also figure 11.

Figure 8 presents a typical example of this transformation. The proximo-distal direction of the nerve is from left to right in the picture. The wide endoneurial spaces on the left can be seen to narrow toward the right and to terminate in a dense, more darkly stained zone, marked F, which has become fibro-

tic. In many cases, the process of condensation continues until a type of sclerosis is reached in which the interstitial tissue has become very dense, more homogeneous and very sparsely settled with cells. Nerve fibers passing through these fibrotic or sclerotic regions are forced into a contorted and meandering course, a condition which in severe cases may perhaps lead to physiological pressure block.

The progressive fibrosis of the distal end of the edematous zone may be correlated with an observation commonly made in early edemas. These show granular tissue debris accumulating near the constriction. This granulated matter may be assumed to consist of degeneration products liberated in the course of ascending traumatic degeneration of the proximal nerve stump. Its localized accumulation just proximal to the level of constriction can be easily accounted for if we assume that the edema is produced by the partial damming up of an interstitial liquid seeping distad. If this fluid carries debris and, upon coming up against the constriction, continues to seep on, even though at a reduced rate, into the compressed region, it is likely that all larger particles suspended in it would be retained at the entrance to the sleeve by the sieve-like action of the suddenly reduced endoneurial channels. In a second phase, the presence of this debris would then stimulate connective tissue formation. While the precise sequence of steps has not been determined, the analogy with inflammatory processes makes this interpretation fairly plausible.

A comparison of figures 9 and 10 with figure 8 reveals some of the story. Figure 9 reproduces an early stage in the formation of the edema, 8 days after arterial splicing of a rat tibial nerve, and figure 10 shows part of figure 9 at higher magnification. One recognizes clearly the zone of the proximal stump to which traumatic fiber modifications have ascended: the axis cylinders are swollen and vacuolated in the manner characteristic of reversible pseudodegeneration as described by Cajal ('28). Edema is already present (E), but at the level we are considering, the endoneurial meshes are filled with a granular debris of obviously local origin, which makes this

zone stand out in the silver preparation as a darker band across the nerve (B). Gradually, however, this interstitial matter will be displaced in a peripheral direction, but it will move no farther than the entrance of the sleeve, where it will pile up. At this more peripheral level, then, the fibrotic transformation illustrated in figure 8 will occur.



Fig. 9 Irritative phenomena in proximal stump of sleeve-spliced nerve (rat R92) 8 days after operation. $\times 48$. Proximal end of sleeve at S.

Fig. 10 Detail of axon vacuolization from zone B of figure 9. $\times 205$.

The formation of a fibrous, and sometimes sclerotic, zone at the distal end of the edema affords an explanation of why edemas persist even in cases in which the arterial constriction is only temporary. If, as we contend, the edema is caused by occlusion of the intraneural spaces, the nature of the occluding agent is immaterial. Compression by the arterial sleeve would initiate the process by narrowing the endoneurial spaces, thus causing the primary edema to form. Then debris would collect and provoke the formation of a connective tissue partition in its place, which, henceforth, would act as a secondary plug of the endoneurial spaces, perpetuating the edema even when the grip of the arterial sleeve on the nerve relaxes, as in some cases it does (compare fig. 3). On the other hand, if we were to assume that the constriction of the sleeve had completely stopped, rather than merely diminished, liquid transfer along the nerve, the accumulation of interstitial debris at the most distal level of the edema could not be explained. We would then find the debris scattered throughout the edematous region much as flotsam in a stagnant pool. In other words, we may consider the fact that the debris always accumulates at this particular level, as a definite indication that the centrifugal seepage in the endoneurial spaces has not been completely arrested but only impeded. This conclusion is fully borne out by observations on secondary and tertiary edemas arising in the more distal course of a nerve.

SECONDARY EDEMAS

The nerve illustrated in figure 11 is a case in point. It is the radial nerve of a chicken, sleeve-spliced to its own peripheral stump with a plasma-filled gap left between the ends, 3 months after the operation. The proximo-distal direction of the nerve is from left to right. The level of the proximal end of the splicing sleeve is marked; it happens to run obliquely. Just proximally (to the left) of this level, one recognizes the heavily fibrotic zone (F_1) of which we have just spoken. Adjoining this zone at the left are the wide spaces of the primary edema (E_1). However, distally (to the right) of the fibrous

zone, inside of the sleeve, we see the structure of the nerve loosen up again, and although this is the portion which was originally compressed, it now contains wide clear spaces. At the far right of the picture, the nerve resumes a denser texture, caused by the fibrous transformation of the plasma clot originally included in the sleeve (F_2). Between the two fibrous zones there is a typical endoneurial edema (E_2). These secondary edemas have been observed in nine nerves in which a



Fig. 11 Secondary edema in sleeve-spliced nerve (chicken C18R; fig. 8) in which plasma clot placed between nerve ends had become fibrotic. Ninety days after operation. $\times 40$. Proximo-distal direction from left to right. Primary (constriction) edema (E_1) with its distal fibrotic zone (F_1) lies proximal to sleeve while secondary edema (E_2) formed behind fibrotic plug F_2 , lies inside of sleeve.

secondary zone of peripheral fibrosis had set up another obstruction to endoneurial seepage. They are never found in well spliced nerves with directly apposed ends, which is in line with the observation that fibrosis does not occur in such nerves.

Like the constriction edemas, the secondary edemas can always be referred to a barrier lying immediately distal to them. To be sure, in our present case, the proximal fibrous partition (F_1) has an edema lying to either side. Yet, its relationship to the distal edema is purely positional, and in no way causal.

Not a single case has come to observation in which there would have been such an edema on the distal side of an obstruction, unless there was still farther distally another plug. Moreover, closer inspection of the secondary edema shows that it gravitates, as it were, towards the distal plug; i.e., there appears more liquid, and the spaces carrying it grow wider, towards the distal barrier. Another edema may appear on the distal side of the second plug if the nerve contains still a third obstruction. A nerve may, therefore, contain a number of barriers in its course, each one causing its own "upstream" edema, comparable to a waterway with a succession of sluices.

Under these circumstances, it is not surprising to find cases in which edemas have formed near a fibrotic plug inside the sleeve even in the absence of a constriction edema farther proximally. Such edemas are in the same position and of the same origin as the ones we have described above as "secondary." They appear in cases in which the splicing artery was wide enough not to cause constriction, but in which prospective obstructions had been placed in the way of the nerve. There is a definite correspondence between the filling of the sleeve and the degree of subsequent connective tissue formation inside of it. As mentioned before, with nerve ends closely apposed, there is practically no fibrosis. There may be some, and sometimes even a considerable amount if a gap is left open between the nerve ends. There is even more if the gap was filled with Ringer's solution, and still more if it was filled with blood plasma. In experiments in which opposite nerves were spliced, one according to one, and the other according to another of these procedures, the correlation between the amount of intraneural fibrosis and proximal edema became quite evident. Thus, in two chickens, C8 and C10, the stumps of the severed radial nerve of one side were spliced end-to-end, while on the opposite side a gap of about 7 mm. was left open between the nerve stumps inside the artery. In these cases, no edema formed in the directly spliced nerve, while conspicuous edemas were present in the nerves with the gaps, which

had developed some islands of connective tissue. In case C16, in which both nerves were spliced with a gap left between the ends, but in which one nerve gap was filled with a blood plasma clot, while the other was left open, the former developed some edema while the latter failed to do so. Likewise, in a pair of nerves in which one was capped with an empty sleeve while the other was capped with an artery filled with Ringer's solution, the latter, after becoming invaded by dense fibrous tissue, provoked an edema while the former, filled with loose tissue, did not.

This last case leads to the consideration of edemas which form in proximal nerve stumps left unconnected peripherally; nerves of amputation stumps belong in this class, and so do, in our experiments, nerve grafts which had been spliced to a proximal nerve stump without being connected peripherally. The peripheral open cut surface became covered by a cap of dense scar tissue such as usually forms over open nerve ends. Again, as a result of this fibrous occlusion of the endoneurial spaces, edematous fluid accumulates right under the fibrous cap, and bulbous terminal edemas arise (fig. 12). The terminal swelling, commonly referred to as bulbous neuroma, consists partly of accumulated interstitial fluid. However, just as was found in the primary edemas, some fibrous replacement of the edematous spaces will gradually set in from the periphery and transform the soft into a more rigid bulb.

What all these secondary and tertiary edemas demonstrate is that none of the observed obstructions — except possibly the terminal one, for which it cannot be decided — shut the more peripheral parts of the nerve completely off from their supply of interstitial fluid; while, at the same time, the strict localization of the edema to the proximal side of any one of the multiple obstructions is evidence that the edematous fluid comes from a proximal source and moves distad. If the edema were of local origin, there would be no reason why it should not form at both sides of the supposedly irritated region. Secondary edemas are particularly instructive evidence

against a supposedly irritative origin of intraneural edemas in that they arise only much later than the primary edemas, at a time when all traumatic effects have long since subsided.

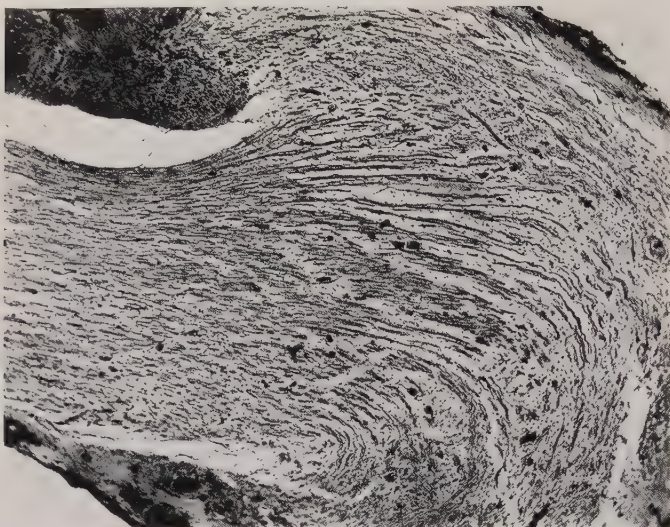


Fig. 12 Terminal neuroma with edema at the free end of a nerve graft which had been sleeve-spliced to the stump of the radial nerve (chicken C8L) 81 days previously. $\times 48$. This nerve contained also a primary edema proximal to the sleeve and a secondary edema within the sleeve.

VASCULARIZATION

The intraneural vascularization in the rat is relatively poor. For instance, measurements of the combined cross sections of the larger blood vessels in the proximal trunk of the sample nerve of figure 1 yield a value of only $1/130$ of the total cross section of the nerve. Sixty per cent of these vessels, moreover, lie at the circumference of the nerve directly under the epineurium. While no detailed study has been made of the changes which these vessels undergo in the course of nerve splicing and nerve regeneration, some pertinent facts are revealed by a survey of the series of cross sections of our preparations. These show a definite decrease in the number as well as the diameter of the recognizable intraneural vessels inside the sleeve, par-

ticularly in the regenerated part of the nerve. Moreover, whatever vessels are evident in the sleeve, are mostly situated at the periphery of the nerve. The edematous region proximal to the sleeve shows possibly a slight increase in the number of blood vessels and possibly also dilation in some of them. A similar dilation and increase are found in the peripheral parts of the nerve distal to the sleeve, where degeneration had originally occurred and might be explained as a response to the degenerative phenomena going on in their territory. However, while there is probably an initial deficit in the vascularity of the sleeve region, this is soon made up for by the establishment of collateral vascularization through the sheath. Within a few days after the operation, the wall of the arterial sleeve is richly vascularized from the epineurial blood vessels of the nerve trunk, and if we take the vascularization of the nerve as a whole, we realize that its interruption and disequilibrium is only a transitory event of short duration. This is an important consideration in connection with the problem of the origin of the edema.

DISCUSSION

From the reported observations, the following facts have emerged. Constriction of a nerve results in edema in that part of the nerve bordering on the proximal end of the compressed zone. The presence of a fibrotic zone inside of a nerve likewise leads to edema, localized at the proximal side of the obstruction. Similarly, a terminal edema may arise behind a terminal fibrous occlusion of the cut end of a proximal nerve stump. The common denominator of these three edema-provoking conditions is neither operative trauma nor chronic irritation, nor vascular stasis, but solely the partial or complete obliteration of the endoneurial spaces through either constriction or plugging. The occlusion, depending on its severity, either reduces or arrests any traffic of liquids that might otherwise have proceeded in the blocked channels. And since the edematous fluid accumulates with striking regularity at the proximal side of the barrier, the prevailing direction of

such traffic must have been centrifugal. This would lead us to the assumption of some pressure in the endoneurial spaces of as yet undefined origin, but with a clearly centrifugal resultant. This conclusion seems cogent no matter what may be the source of the fluid itself. The latter may be a regular constituent of the nerve or it may arise only under the conditions of the experiment. In either case, its "upstream" accumulation presents a problem of its own.

However, before committing ourselves definitely to the blocking of endoneurial channels as an explanation of these edemas, we must consider some alternative interpretations.

There is the possibility of a vascular origin of the edema — transudation from occluded vessels as in common edemas; more specifically, from arterial vessels, because of the confinement of the exudate to the proximal side of a constriction. The facts, however, seem to disprove this assumption. First of all, the vascular stasis following the operation is of very brief duration. As described above, collateral vascularization through the sleeve is established within a few days. Stroebe (1893) reports restitution of circulation through a compressed and crushed nerve segment of several millimeters within 48 hours. Blood pressure equilibrium is thus restored to the nerve as a whole before the edema has even reached its full extent. Moreover, nerves which have simply been cut and either left severed or adequately reunited without constriction develop no edemas, although their vascular system has suffered the same initial interference as that of the edematous nerves. In appraising the situation, one must never lose sight of the fact that the nerve edemas in our cases have persisted for many months, long outlasting any vascular instability that may have existed in the beginning. However, perhaps the most convincing evidence comes from the secondary and terminal edemas, which form without even transitory constriction of vessels and in which, in fact, the vascularity beyond the edema is more abundant than it is in the edematous zone itself. The fibrotic nuclei in the nerve, behind which edemas are commonly found, are well vascularized and offer no obstruction to

blood flow. And the same applies to the fibrous scar over a free nerve end, which, nevertheless, may become the seat of a terminal edema.

Discounting the thesis of a vascular origin of the edema, we turn to the possibility that the nerve fibers themselves may have exuded the edematous fluid as a result of temporary trauma or permanent irritation. The problem poses three separate questions: First, do nerve fibers exude fluid? Second, if so, do they do so normally or only under pathological irritation? Finally, could this fact account for persistent edemas of the observed localization? For all we know, nerve fibers may very well discharge some liquid. But this in itself would not explain the locally confined edemas; for if fluid oozes from the fibers at all levels, one would have to postulate some additional factor responsible for the local accumulation of ubiquitous exudate — which would bring us back to the concept of endoneurial flow. The alternative would be that the fibers discharge fluid only at the levels where we later find the edemas.

Let us consider this latter possibility of a local formation and local confinement of the edemas more concretely, taking the sleeve-constricted nerves first. There is Wallerian degeneration in the nerve below, and some ascending traumatic degeneration in the nerve above, the constriction. Degenerating nerve fiber segments contain liquid vacuoles and swell considerably, but from the fact that the packing of the resulting Schwann tubes (Buengner's cords) remains compact (compare fig. 4D in Weiss and Davis, '43), we may infer that no excessive amount of this liquid leaves the tubes. The peripheral stump is swollen (figs. 1, 2), but not edematous. The ascending changes of the proximal stump, on the other hand, are of a somewhat different nature. Here, many axis cylinders go merely through a process of varicose swelling without ever disintegrating. These symptoms, described in detail by Cajal ('28) and observed by Speidel ('35) in living fibers after irritation, are exemplified in figures 9 and 10. Conceivably, these ascending traumatic lesions may involve changes in the

permeability of the fiber sheaths which would let some of the axonal fluid escape into the interstices. In this connection an observation of Stroebe (1893) on rabbit nerves that had been pinched through with a clamp seems worth citing (p. 195; translated from the original German): "The whole central stump . . . shows a diffuse pale blue tint in Anilin Blue, clouding all histological details; this diffuse stain is obviously due to the infiltration of the stump with a protein-containing serous fluid, which must be regarded as a product of the traumatic lesion." Stroebe saw some of this infiltration also in the peripheral stump near the injury. Might we not, then, be confronted here with an incipient edema? According to Stroebe's further description, no. For since, according to his report (p. 200), these infiltrations disappear on the third day, they can hardly be identical with the persisting edemas of our cases. Moreover, the absence of edema in merely transected or transected and well spliced nerves, in which the initial trauma was fully as great as in the edematous cases, as well as the late appearance of secondary and terminal edemas in the absence of all trauma, seem to eliminate trauma as a possible source of our edemas.

Since we have been stressing the persistence of the edema as an argument against its vascular or traumatic origin, it should be reiterated that the edema really is permanent (within the duration of our experiments). This statement does not run counter to our observation reported above, that firm connective tissue gradually invades the edematous spaces, since the latter process remains confined to the peripheral layers of the edematous bulb, while the core remains liquid (figs. 8, 11).

For the outlined reasons, it seems indicated to abandon the concept of a local origin of the edematous fluid in favor of the assumption of its ubiquitous presence in the nerve with merely preferential accumulation in physically predisposed locations. According to this view, the edema would be but the normal fluid content of the endoneurial spaces, dammed up by

an obstruction. This still leaves the questions of the source of this fluid and of the mode of its distribution unanswered.

There are three possible sources. Firstly, it may be cerebrospinal fluid draining from the subdural space directly into the endoneurial spaces. Secondly, it may be a secretion of the nerve fibers themselves. Thirdly, it may be a mixture of both. There appear to be no anatomical barriers that would prevent cerebrospinal fluid from spreading from the subdural space along the surfaces of the nerve fibers into the peripheral nerves way beyond their intraspinal course, for which such communication has been established, even though the amount of this capillary drainage may be negligible as compared to the volume escaping into the venous sinuses and lymphatics (Weed, '22). Experimentally, the passing of substances injected into the peripheral nerve up through the interstices and on into the subdural space has been demonstrated (review in Speransky, '35). The fact that the excess pressure used to force the injected mass into the nerve may reduce the physiological pertinence of such experiments, does not detract from their value in demonstrating the existence of a pathway between subdural and endoneurial spaces. Consequently, it is at least possible that the liquid base of the endoneurial edemas may be derived from cerebro-spinal fluid. In view of the apparently high protein content of the edemas, in contrast to the known low protein content of the cerebro-spinal fluid, the admixture to the latter of some peripheral product would seem likely. This addition may come from the nerve fibers. Possibly even the whole of the endoneurial fluid is exuded from the nerve fibers. But since there are practically no facts known on which to base speculation, we prefer to leave these questions open.

Turning now to the second problem, that of the distribution of the endoneurial fluid, one fact seems to emerge with certainty: the fluid cannot be stagnant, but must be in centrifugal motion. Only thus can we account for the characteristic position and shape of the edematous swellings. The existence of such motion raises the question of the motive force. Under

normal conditions, the fluid is presumably resorbed from the peripheral channels at the same rate at which it is fed into their proximal ends. Any reduction in the width of the channels, e.g., by constriction or fibrosis, leads to proximal damming up, which means that the fluid continues to be fed into the lines although the rate of its peripheral disposal is reduced. This fact explains the "upstream" position of the edema, but not its shape. For equalization of pressure in the dammed up pool would tend to widen the "upstream" channels uniformly rather than just in the region immediately before the obstruction. A pear-shaped widening of the kind seen in our cases could arise on purely hydrodynamic principles, but only in currents of such high velocity that it can have no bearing on our problem, which concerns seepage rather than streaming. Only a constant force pressing the fluid steadily distad can explain why the accumulated edema does not gradually flow back into the rest of the nerve. In view of the large total inner surface of the endoneurial meshwork, the resistance to flow in it must be considerable, and, therefore, the postulated driving force must be correspondingly high. The hydrostatic pressure of the cerebro-spinal fluid (order of magnitude: 100 mm. water; Weed '22), for instance, would be insufficient.

We need some mechanism that would squeeze or massage, as it were, the endoneurial fluid down its channels. In this connection the pulse wave deserves consideration. If we visualize the nerve with its sheath as a closed, relatively incompressible system, the increase in blood volume in the epi- and perineurial arteries during the systolic phase of the pulse would necessarily displace an equivalent amount of extravascular fluid. During the diastolic phase, this fluid could return. Now, if the systolic and the diastolic waves were symmetrical, a regular tide, even though only of small scope, would result. However, the asymmetry of the pulse, with the systolic pressure wave developing faster than the diastolic decompression wave, may yield a resultant pressure, and consequently shift, of the surrounding liquid in the direction of the fast phase, that is, peripherad.

The physical possibility of such an effect can be demonstrated by means of a simple model (fig. 13). The point to be tested is whether a rhythmic succession of asymmetrical compression-decompression waves passing down an elastic tube can produce translatory movement of liquid in a second tube in lateral contact, but without communication, with the first if both are enclosed in a common sheath. In the model, the outer sheath

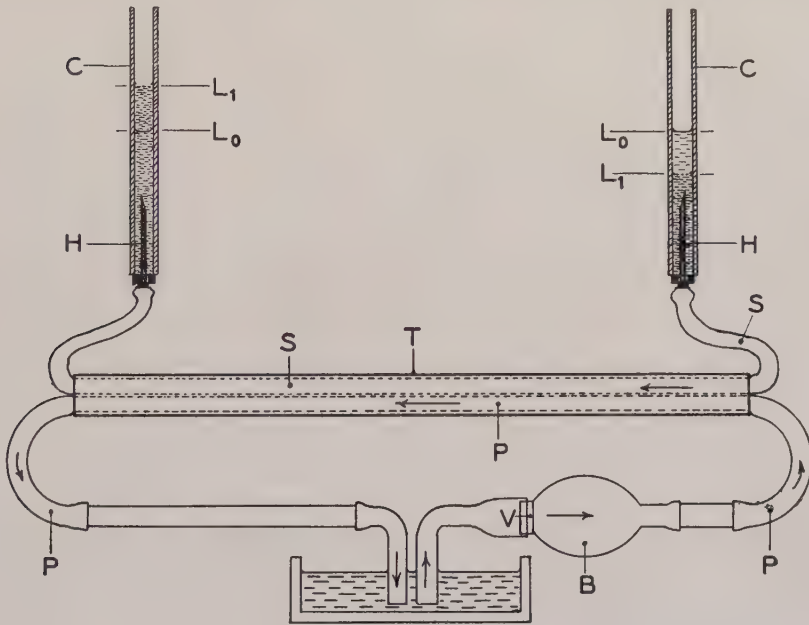


Fig. 13 Model of hypothetic effect of the pulse wave on the shift of fluid in the adjoining nerve spaces. Explanation in text.

of the nerve is represented by a glass tube (T), through which are drawn two rubber tubes (P and S) to serve as conductors for the primary, or pumping, circuit and the secondary, or indicator, circuit. The former is to represent the perineural arteries carrying synchronized pulse waves, while the latter represents the aggregate endoneural space. Both were pulled through the glass tube under stretch so that they partly compress each other. Any dilation of the primary tube will thus

encroach upon the secondary tube. The primary circuit is filled with water by means of a plumber's bulb (B) with an intake valve (V) controlling the direction of flow. Compression of the bulb drives a water column in the direction of the arrow and thus imitates a systolic pulse. Upon release, the bulb fills itself through the valve, reproducing the diastolic phase. The indicator circuit (S) is wholly symmetrical and communicates with two terminal glass tubes (C) through hypodermic needles (H) of identical gauge. When in equilibrium, the water levels (L_0) are the same in both glass tubes.

When gentle pressure is applied to the bulb, the primary tube (P) is dilated, and the secondary tube (S) correspondingly compressed, which registers as a rise of the water levels in both indicator vessels (C). If, however, the bulb is compressed quickly, the rise of the water level is greater in the distal than in the proximal vessel. Evidently, there has been a shift of water in the secondary system in the direction of the primary current, the latter exerting a pumping action on the adjacent channel. While the effect of a single pulse is small, rhythmic repetition at suitable intervals soon leads to a conspicuous difference between the two water levels (L_1), provided the rate of backflow (determined by the width of the hypodermic needles H) is slow enough to permit each successive pressure difference to build up on top of a residue of the preceding ones. Reversal of the flow in the primary circuit (by inserting the pump in the left branch), of course, reverses the effect in the secondary system. This test of reversibility is important in order to eliminate errors due to possible intrinsic asymmetries within the secondary system itself. If the two end tubes were plastic and closed up at the resting level L_0 , the excess water in the distal one (corresponding to the water column L_0-L_1) would obviously cause local inflation; in other words, reproduce the edemas of our experiments. Without obstruction, in the living object, the excess would simply drain off into the periphery instead of flowing back, as it does in our model owing to gravity.

The model presents merely a physical possibility. How close it comes to physiological reality, is another matter which can be decided only by further experiments. Even though the individual perineurial vessels are relatively small (p. 510), their synchronized pulsation would summate their effects. Moreover, the cumulative action of incessant pulsation over long periods of time would tend to amplify the imperceptible effects of the single beats.

In conclusion, our contention is that the endoneurial spaces are filled with a liquid which is constantly propelled, even though at a slow rate, in a proximo-distal direction. The vascular pulse is suggested as a means of such translatory motion. Obstruction of the channels of this flow would impede its peripheral drainage and lead to local damming up in the form of bulbous edemas. That even then a certain amount of the fluid continues to seep on beyond the obstruction, is evidenced by the secondary and tertiary edemas forming before blocks situated farther distally. This explains why debris accumulates at the near site of a compression: the liquid continues downward, while particles too large to pass are retained as by a strainer.

Its prevailing centrifugal course would make the endoneurial fluid a poor vehicle for centripetal transport of particulate matter in the nerve. This gives added, though indirect, support to the theory that such afferent transport, especially of neurotropic viruses, proceeds within, rather than between, the nerve fibers (Howe and Bodian, '42; also review of earlier literature).

The endoneurial fluid seems to play a peculiar role in nerve regeneration. Growing nerve sprouts as well as sheath cells extend only along interfaces, and there are increasing signs that the nature of the interface affects the rate and direction of the advance (Weiss, '41 a). It now appears that impregnation with endoneurial exudate makes a tissue more pervious for sheath cells and nerve sprouts. The presence of this medium in the gap between severed nerve stumps seems to create

exceedingly favorable conditions for regeneration. When two nerve ends are spliced with a hermetically sealing sleeve of artery (Weiss, 41 b, '43), the endoneurial fluid apparently exudes into the space between the nerve ends and forms a bridge which can be easily traversed by the regenerating sprouts without branching and in perfectly straight orientation. At the same time it suppresses the formation of fibrous connective tissue. The high liquid content of the matrix in which regeneration takes place in such regions is in striking contrast to the dense fibrosity of ordinary nerve scars. When a nerve is simply cut, this medium presumably seeps from the wound into the surroundings and is dissipated. However, if the nerve ends are sheathed in by a sleeve, the fluid collects in the gap where it exerts its beneficial influence on the regenerating sprouts. Its coagulation seems to establish interfaces peculiarly suited for sheath cell and nerve fiber application; it may also, by coating existing surfaces, serve as a binding between the nerve fibers and their non-neural surroundings. Whether it has, in addition, nutrient or metabolic significance, is an open question.

These observations have recently received strong support from tissue culture studies (as yet unpublished). When spinal ganglia of chick embryos are cultured along the surface between a cover glass and an inorganic liquid medium, one often notices a substance seep from the ganglion and spread along the solid-liquid interface. When fixed and impregnated with silver, it forms a very fine reticulate precipitate of much the same appearance as was described above for the edema. From its staining reaction, we may again infer its protein nature, and this time we can definitely identify it as coming from the nerve culture. Later, when nerve fibers grow out, they seem to hold themselves within this film and rarely proceed beyond its borders, and even when they do, it is not certain that they are not coated with a submicroscopic layer of this material. These experiments seem to indicate, therefore, that nerve fibers in "liquid-medium" tissue culture move preferentially, if not exclusively, within an exudate of their own making. Al-

though the identity of this substance with the one recovered from the endoneurial spaces in the form of edema remains to be demonstrated, the similarity of behavior and histological reactivity is suggestive.

In practical respects, the significance of the endoneurial fluid in nerve regeneration raises the question of whether what we have described for the nerves of the chicken and the rat, applies to human nerves as well. Dustin ('17) has given incidental mention to edemas observed in human nerves constricted by scar tissue. Judging from his description, the resemblance to our animal cases is close. He states (p. 127; translated from the original French): ". . . The laminated sheaths are distended by edematous fluid which after fixation and impregnation can be recognized as a fine albuminous precipitate. This edema dissociates the constituent bundles of the nerve trunk; it first infiltrates into the meshes of the connective tissue of the nerve, later penetrates between the fibers themselves which it soon separates from one another." By way of interpretation, he proposes (p. 128) that "the lymphatic circulation of the nerve is independent of the general lymphatic circulation," and "the lymphatic circulation of the nerve seems to be in close connection with that of the cerebrospinal fluid." Following Sabin's ('16) precept, it would seem inadvisable to designate the nerve fluid as "lymph," but otherwise, Dustin's remarks seem highly pertinent.

SUMMARY

Chronic constriction of a nerve by an arterial sleeve produces persistent edemas in the endoneurial spaces just proximal to the compressed zone. This phenomenon has been studied histologically in thirty rat and chicken nerves preserved from 1 to 17 weeks after the operation. Similar edemas form at the proximal side of any kind of obstruction within a nerve (e.g., a fibrotic plug in the course of the nerve or a fibrous cap over the blind end of a proximal nerve stump). These edemas are neither of vascular nor of irritative origin. Evidence has been presented to show that they result from the damming up of

fluid normally present in the endoneurial spaces and seeping distad. The pulse wave may hypothetically be assumed to furnish the propulsive force for this seepage. The endoneurial fluid seems to have special significance in nerve regeneration, inasmuch as it forms a superior growth medium for the outgrowing sheath cells and nerve sprouts and also has anti-fibrotic properties.

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THE ADRENAL LIPIDS OF MICE WITH HIGH AND LOW MAMMARY GLAND TUMOR INCIDENCES

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TWO FIGURES

INTRODUCTION

The interest in the rôle of the cortical lipids of the adrenal gland has been renewed since Hoerr ('36) pointed out that the masked lipid, a constant constituent of most tissues, has in all likelihood the same function in the adrenal gland that it has in other tissues. He concluded that the visible lipids in the adrenal cortex are concerned primarily with the specific function of the gland. These visible adrenal lipids are histologically demonstrable in sections of the gland. The object of this investigation is to study and record the amount and distribution of the visible lipids in the adrenal gland in relation to mammary gland tumor incidence. It has been felt that the lipid cholesterol, might play a vital rôle in the production of tumors. At what age the lipids may exert their influence on the incidence of mammary tumors is not known. In order to obtain this information a basic investigation was begun. A series of studies have been undertaken to record the amount and distribution of cortical lipids of the adrenal gland in a series of different age groups throughout the life span of the individual, for mouse strains of high and low mammary tumor incidences.

The fundamental architectural plan of the mammalian adrenal gland is similar in all species. The gland consists of two components, the outer cortex and the inner medulla.

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From its border proceeding inwards the cortex consists of its capsule and three zones: glomerulosa, fasciculata and reticularis. These terms are based chiefly on the arrangement of the cells, and roughly correspond to the presecretory, secretory and postsecretory zones of Bennett ('40). The idea that the cortical cells grow from the peripheral region and differentiate inward has been universally accepted.

The adrenal gland of the mouse conforms in general to the typical mammalian plan and differs only in few histological details. The current opinion is that the zona reticularis in the mouse is diminutive in the adult and absent in early life. In its place there is at first an X-zone, transitory in nature but distinctive, the real origin of which is still a controversial point. This transitory or X-zone is more conspicuous and persistent in the female than in the male. On its disappearance during pregnancy and in the adult the classical zona reticularis seems to appear. The present observations show a variation in the widths of this zone in different strains of mice. The transitory or X-zone has not been definitely reported in the rat, cat, guinea pig, dog, cow or sheep. In man the X-zone is present in the foetus where it is large. It involutes rapidly, however, after birth and has been called the "rudimentary androgenic zone." Roaf ('35) considered the interlocking zone at the cortico-medullary region in the rabbit homologous to the transitory zone of the mouse. As to its origin, Waring ('35) contended that it came directly from the cortical embryonic anlage. McPhail ('42) traced it back to the fifteenth day of foetal life when the glomerulosa and fasciculata cells are differentiating. McPhail believed that it differentiates directly from the fasciculata but has not given a clear evidence for his belief. As to the nature of the transitory zone, theories have been advanced concerning its cortical or androgenic nature. The data, however, so far advanced have not been conclusive. The concern here is its lipid content and relationships. Howard-Miller ('27) believed that its involution in the female was accompanied by fatty degeneration. But Deanesly and Whitehead ('33)

proved in their material at least that its involution in the female occurred without this phenomenon. Daughaday ('41) reported that the zone in the dilute brown strain virgin female mice persisted for over 200 days and that its regression was accompanied by vacuolization, while in the C57 Black strain female mice it underwent complete regression by 100 days without vacuolization. Preston ('28) showed, after thyroxin administration, a marked increase in the amount of lipid in the cells of this zone. Gersh and Grollman ('39) reported small, lipid droplets in the X-zone after administration of thyroid extract.

The cortex is rich in lipid material, and secretes cortin, which is essential for life. The medulla secretes adrenalin which, although important, is not essential for life. Chemical studies have shown that the vital cortical substance of the adrenal gland can be extracted with the lipids. H. S. Bennett ('40) claimed that the lipids found in the adrenal gland may either be the raw substance from which the hormone is actually made or the vehicle in which it is stored. He showed that, in the cat adrenal, the extent of distribution of ketone compounds, the active ketone sterols, is confined to the secretory zone, or zona fasciculata. He also demonstrated that cholesterol, a possible precursor of the adrenal cortical hormone, is present in the secretory zone of the adrenal gland in large amounts. He went on to suggest that the cortical hormone may be actually manufactured in the secretory cells, which are large and contain visible lipids in fine droplets.

There are two methods of approach in determining the presence or absence of cortical adrenal lipids. One method is the purely chemical analysis. The other method is the histological or histochemical approach. Recent developments on a method for assaying lipids in fluids and tissues make a chemical analysis possible on a small amount of tissue (Bloor, '28, '29). An analysis by this method has shown the adrenal gland to contain fatty acids, phospholipids, and cholesterol (Brown, Knouff, et al., '37). The second or histochemical method consists of demonstrating on histological sections the

total visible lipids by Sudan Orange staining and the cholesterol content by the Schultz chemical reaction test. In recent years the histochemical method has been perfected. Romeis ('29) modified the old Sudan Orange solution to form a colloidal one and claimed it to be more reliable than the former type. Kay and Whitehead ('37) have evaluated the older and newer histochemical techniques and have shown that the use of Romeis' Sudan III in colloidal solution is superior to the old methods for demonstrating total visible lipids. For detecting cholesterol in histological sections the only acceptable method is the "Schultz positive" test. Lison ('36) in his book "Histochemie Animale" has reviewed the whole subject and has done much towards producing a better understanding of the problems involved. Knouff ('41) in his correlation of chemical and exact histochemical studies (both for total visible lipids and cholesterol content) on guinea pig adrenals analyzed chemically one adrenal and histologically the other adrenal of the same animal. He reported that for all practical purposes the two methods agreed.

MATERIAL AND METHODS

The materials used for this investigation are adrenal glands from various inbred genetic strains of mice maintained by the Roscoe B. Jackson Memorial Laboratory. Six strains have been selected for observations: (1) the C57 Black strain, (2) the ce or extreme dilution (an allele of albinism), (3) the dilute brown or dba strain, (4) the C3H strain (agouti in color), (5) the A-albino strain and (6) the N strain (piebald) in color. The incidence of mammary gland tumor of these strains is high for strains dba and C3H, and low for strains C57 Black, ce and N. For A-albino strain the incidence is high for female breeders and low for virgin females. Pairs of these six strains were obtained and bred. The offspring produced were killed at various ages. The survey consists of the study of the adrenal glands of these strains at stated age intervals beginning with 34 days of age and continuing at monthly intervals through 18 months. Special em-

phasis will be given to the 34-day, 2-month, 3-month, 6-month, 9-month and 12-month age groups, since work on the adrenal gland at this laboratory indicates that these are crucial periods in the life of the individual. This report deals with the "1-month" or 34-day age group and involves approximately 150 individuals distributed among the six strains.

The adrenal glands were fixed immediately at time of killing in 10% neutral formalin. The adrenal gland of the mouse is very small. In order not to pinch it when taking it out of the body the following procedure was adopted. The adrenal was left attached to the pole of the kidney and the tissue cut was made through the polar region of the kidney. By means of holding on to this kidney tissue the adrenal gland was transferred to the fixative. The same procedure was used in subsequent histological steps thus avoiding mechanical injury to the adrenal. Sections were made by the freezing method and were cut uniformly at 15 micra. One adrenal gland was sectioned for Sudan staining and the other of the same animal was sectioned for the cholesterol test. The cortex was found to be of the same width in both glands from one mouse. Deanesly ('28) and Whitehead ('33) also found this to be true. For revealing the total content of visible lipids the Romeis' Sudan III method was used. For cholesterol content the method employed was the application of the Liebermann-Burchardt cholesterol method known as the "Schultz positive" reaction test, so called because the latter investigator applied the method to histological sections. Kaufmann and Lehmann ('29) recommended this test as the only reliable histochemical test for cholesterol. When the reaction is positive a bluish green color appears indicating that cholesterol is present. The color itself does not represent cholesterol but is a resultant reaction in the presence of that substance. If no cholesterol is present no color is apparent. The test is never positive in the absence of cholesterol but it may be negative when cholesterol is present in extremely small amounts.

To obtain the amount and distribution of cortical lipids a method of measurement that would be simple and accurate was desirable. It was decided that the relative width of the lipid band of uniform density would be a good criterion for measuring the amount of lipids. All sections of a gland were examined microscopically to study the variation in width of the cortex. Representative sections, where the widths of the cortices were the least variable, were selected for measurement. These were transverse sections and came from the middle of the gland. The width of the whole cortex and the width of the lipid bands within this cortex were measured with an ocular micrometer at one place in the section and were recorded separately. The location of the selected sections was the minor axis, at right angles to the major axis midway between the poles. The cortex is fairly constant in width on the minor axis, but varies most at the poles themselves. This procedure was followed for all glands of the different strains. Two readings for each width were taken and at least three representative sections of each gland were used for the readings. The widths were averaged to give the individual's index width in terms of the number of ocular micrometer units. Each unit actually represents 0.001 mm. calibrated by a 1-mm. stage micrometer. As the magnification was kept constant a comparative study can best be made on the number of ocular micrometer units. Where the density of the lipid was not uniform the zones of visibly different densities were measured separately. The density of the lipid was estimated by the size and number of the lipid droplets in a unit area. The grades of density were arbitrarily represented by plus characters from one plus to five plus ranging from a trace of lipid to heaviest lipid density.

OBSERVATIONS

The adrenal lipids, of the six strains, at age of 34 days, showed a variation in relative amount. The width was clearly less in some strains than in others. Moreover the density of

the lipid band was not uniform throughout its width but was made up of zones of different densities. In the zona glomerulosa the lipid density was of medium grade, or ++ and +++, and will be referred to as Sudanophile zone I. In the zona fasciculata the lipid density was heaviest. This heavy lipid-laden band will be referred to as Sudanophile zone II and its density grade signified as +++++, ++++++. In some strains the innermost border of this zone was irregular or streaky (e.g. slightly so in the N strain and definitely so in the dba strain). The cortico-medullary or juxta-medullary area was lipid-free in some strains and faintly dotted with few small scattered lipid droplets in other strains. The lipid density of this area was recorded as O, + and called Sudanophile zone III. Whether this lipid-free zone coincided with the morphological X-zone alone or with the innermost margin of the fasciculata together with that zone was not clear.

COMPARISON OF THE SIX STRAINS

Lipid amount and distribution

The six strains were compared for the amount and distribution of cortical lipids. The Sudanophile zone I with a medium density of lipid, ++, +++, was found in all strains. The width of this zone varied from 2 to 6 ocular micrometer units. In four strains this zone was very slightly wider in the male than the female. Sudanophile zone II was the widest and the heaviest in lipids. The average width of this zone varied greatly among the six strains. In order of decreasing number of ocular micrometer units the average male widths of the six strains were as follows: 36 for C57 Black strain, 34 for N strain, 28 for ce strain, 24 for A-albino strain, 16 units for C3H strain and 14 for the dba strain. The female widths, with the exception of strain ce, were slightly narrower than the males. It will be noted that the widths of the Sudanophile zone II for both sexes of strains C3H and dba was less than one-half that of the C57 Black strain.

Sudanophile zone III is the practically lipid-free zone. This zone measured for the strains 1 to 9 units for the males and 4 to 10 units for the females. The widths in ocular micrometer units of zones I, II and III for the different strains of each sex are given in table 1. The males of strains C57 Black, N and A-albino had a lipid-free zone of only 1 unit in width compared with four units for the females. For the other strains — ce, C3H and dba — the male widths for zone III are 4, 3 and 9 units and the female widths, 6, 10 and 10 respectively.

TABLE 1
Actual widths of the Sudanophile zones.
34-day-old group

ZONES	LIPID DENSITY ¹	PURE STRAINS													
		C57 Blk		N		ce		A-alb		C3H		dba		dba	
												norm.		theelin	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
		OCULAR MICROMETER UNITS													
I	++ ++++	5	2	5	3	2	2	6	3	4	5	5	3	5	1
II	+++++ ++++++	36	36	34	34	28	35	24	23	16	16	14	12	24	27
III	O +	1	4	1	4	4	6	1	4	3	10	9	10	4	5 ²

¹ Density is represented by + signs; low grade by +, medium grades by ++ and +++, heavy grades by ++++ and +++++, no lipid by O.

² Between this lipid-free zone and the medulla, interlocking with the latter, there is an irregular band averaging in width fourteen micrometer units and containing low density lipid.

For a comparative study of the amount and distribution of the Sudanophile material the zones were estimated on a percentage basis against the width of the total cortex as 100%. This was done for each sex and strain. The results are given in table 2. The percentage width of the male Sudanophile zone I varied among the strains from 11 to 19% and from 4 to 16% for the females. The per cent width of the Sudanophile zone II varied among the strains as follows. The

males per cent widths were: 87 for C57 Black; 85 for N strain; 82.4 for ce strain; 77.5 for A-albino strain; 69.7 for C3H strain and 50% for the dba strain. The female per cent widths were, with the exception of strain C3H, slightly less than that of the males. The difference in the per cent widths of C3H was 18% less in the female than the male — a marked difference. The female lipid band is one-half the cortex width while the male's is two-thirds. This is due to the much wider zone III of the female. Comparing the two extreme strains, the dba and C57 Black, the cortex of the dba was lipid-laden only to the extent of half its width while the C57 Black's was lipid-laden to the extent of seven-tenths of its width.

TABLE 2

Per cent widths of the Sudanophile zones to the whole cortex

ZONES	LIPID DENSITY	C57Blk		N		ce		A-alb		C3H		dba	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
I	++	11.0	4.5	12.5	7.3	5.9	4.6	19.3	10.0	17.3	16.1	17.9	12.0
	+++												
II	++++	87.0	86.5	85.0	83.0	82.4	81.6	77.5	76.7	69.7	51.7	50.0	48.0
	+++++												
III	O	2.0	9.0	2.5	9.7	11.7	13.8	3.2	13.3	13.0	32.2	32.1	40.0
	+												

The relative widths of the "lipid-free band" or zone III in terms of per cent width of the cortex are as follows: for strains C57 Black, N, A-albino and ce, 2 to 3% for the males and 9 to 13.8% for the females. The per cent width of this zone for the C3H strain was 13 for the males, and 32.2 for the females while for the dba strain it was 32.1% for the males and 40% for the females.

An attempt was made to show graphically the relative proportions of the three zones: Sudanophile zone I and II and the lipid-free zone III. These are given in figure 1 for each sex and strain in a subdivided percentage bar chart. The rectangular bars of the same width and the same length are constructed on the same base line. The entire lengths of

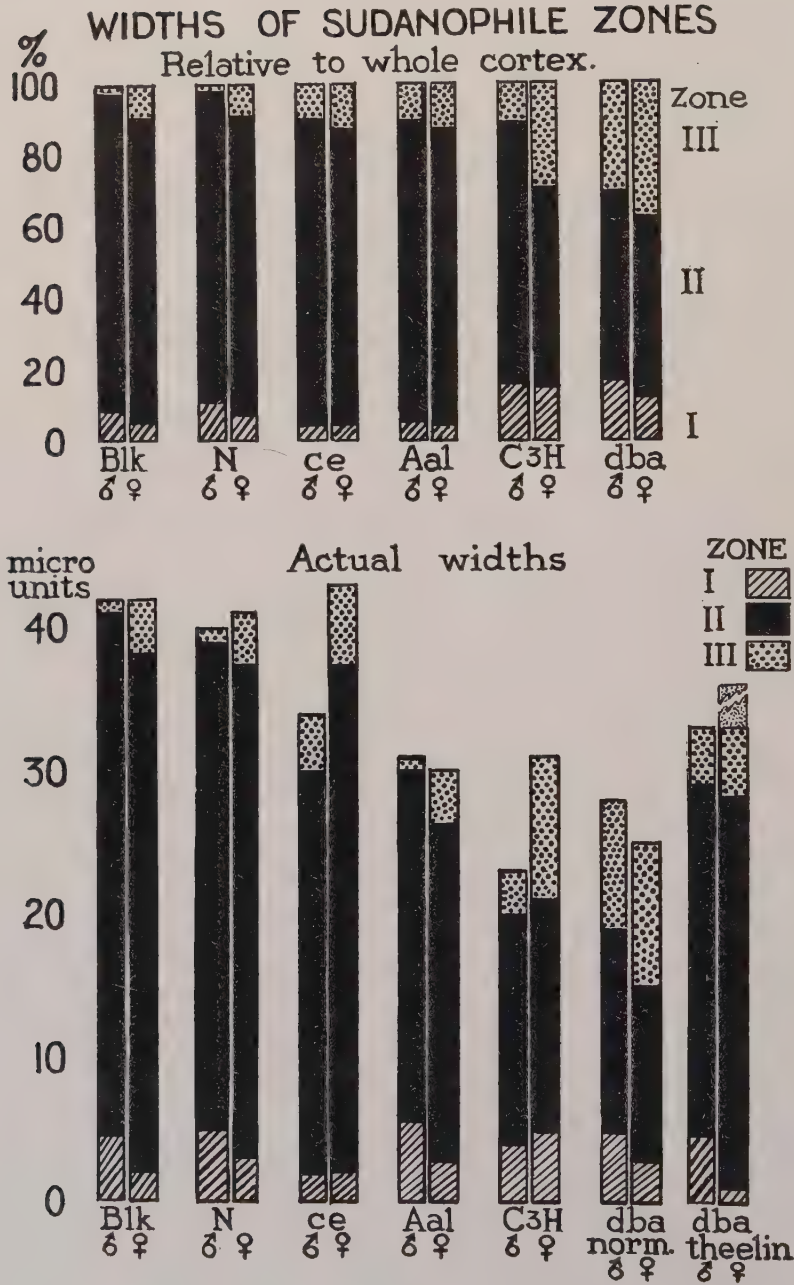


Figure 1

bars represent the widths of the cortices as 100%. Each bar is divided into three smaller bars or segments which represent the three Sudanophile zones I, II, III. These are arranged in the order of their position in the actual cortex. The size of each segment is dependent on the per cent of the total cortex it represents. The segments representing the three zones are represented by designated bars that make up the whole. In the lower half of figure 1 a second bar chart represents the actual or absolute units of each zone. The strains are arranged from left to right in order of decreasing amount of total lipids or Sudanophile material. It is clearly seen that the C57 Black strain contains the greatest amount of lipid material while the dba strain possesses the lowest amount. The male has actually and relatively more lipid material than the female. This is true for each strain with the exception of ce strain where the two sexes are the same on the per cent basis although actually the ce female has slightly more lipid than the ce male.

Cholesterol distribution

The Schultz positive test for the presence of cholesterol was applied to the histological sections. The presence of cholesterol was observed to be distributed along the same areas as the Sudanophile material. The variations in cholesterol density were practically the same as the variations in the Sudanophile density. There was no significant interference of neutral fats. This suggests that the cortical lipids are to a large extent cholesterol.

A HIGH AND A LOW INCIDENCE STRAIN CONTRASTED

In order to see whether the strain of high mammary tumor incidence is really different from the strain with the low incidence in its amount of lipids, strain C57 Black was contrasted with strain dba. Frequency distribution curves of the strains have been plotted and are given in figure 2. The abscissae represent the classes for the amount of lipid ma-

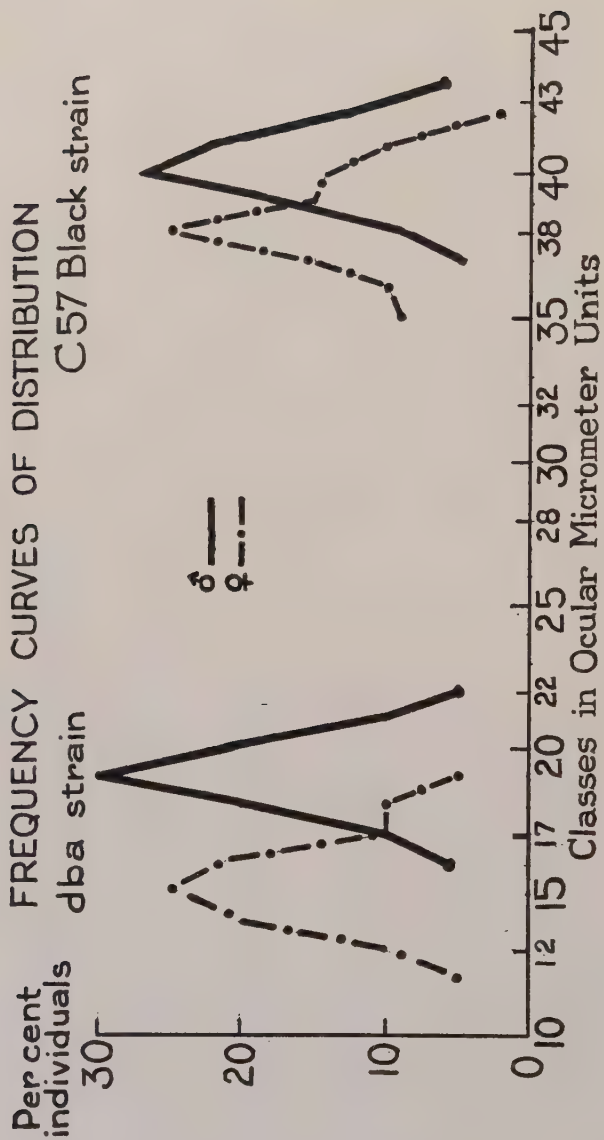


Figure 2

terial which includes Sudanophile zone I and zone II determined by their widths and expressed in terms of ocular micrometer units. The ordinates represent the per cent of individuals in each class. The classes range from 10 to 45 units. The C57 Black classes are distributed between 35 and 43 units while the dba between 11 and 22 units. For each strain the frequency curves of the two sexes slightly overlap each other indicating no large sex difference. The two strains are wide apart, so wide that there is no question of their being different and distinct. It can thus be said that the two strains are definitely different with respect to the amount of total visible lipids and amount of cholesterol. It is worth mentioning once more that the dba strain is the only strain of the six with a distinctly streaky or irregular zone II.

Minor features of the strains

Strain C57 Black had in both male and female large lipid droplets (grouped from one to five droplets) located irregularly on the medulla side bordering on the juxtamedullary region. These lipid groups stood out clearly, the droplets being larger than the droplets found in the cortex proper. In strain A-albino the female zone III interlocked with the medulla. The lipid groups described above for the C57 Black were not observed in this strain. The margin of Sudanophile zone II which bordered on the lipid-free zone III was slightly irregular for the female of strain N. Both sexes of dba strain showed a finger-like or streaky zone II. In addition the female zone III exhibited small lipid droplets of low density in its innermost region bordering directly on the medulla, forming a "rim" about $1\frac{1}{2}$ ocular micrometer units in width. The female C3H lipid-free zone III interlocked somewhat with the medulla. Both sexes of ce strain showed a lipid-free zone III with its inner border projecting in a mycelium-like manner through the medulla. In addition the female had in the lipid-free zone III scattered groups of two or three large lipid droplets.

ACCESSORY ADRENALS

Small accessory adrenals were found more frequently in the C57 Black strain than in any other. Two or three of these accessory adrenals were observed approximately as many times near the right adrenal as near the left adrenal gland. Usually those at the right side were in males and those at the left in females. These small accessory glands were sectioned and stained and were found to have cortical lipids. No attempt was made to add the amount of lipid in these accessory adrenals to the total for the individual, as the strain with the greatest number of accessory glands was also the strain with the greatest amount of cortical lipid in the normal gland.²

EFFECT OF THEELIN ON THE dba STRAIN

To see if the lipid amount of the dba strain could be made experimentally to approach the amount of the C57 Black strain, theelin was given to a small group of males and females of the dba strain. All the individuals were 27 days old at the beginning of the theelin administration. Two males and two females were given two daily doses of 0.4 cc. of theelin by injection under the skin, and two females and two males were given by similar injection several daily consecutive doses, the first two of 0.4 cc. and the next five of 0.5 cc. each. All treated animals were killed at 37 days of age and ten related control dba individuals of 37 days old were, at the same time, also killed.

² Closely resembling the C57 Black in the incidence of accessory glands was the leaden strain, L, and the C57 brown strain. Although not reported here at length, preliminary studies on these two stocks show a close resemblance of their lipid distribution and amount to the C57 Blacks. It is interesting to note that they were both derived many generations ago from the C57 Black by mutation and selection. The A albino had a number of accessory adrenals while the dba strain seldom had them. If they occur at all in dba mice they consist of only one (small or very small) on the left side and the individual was more often than not a male. The statistical significance of the relation of accessory adrenals to one or the other side in relation to sex is not clear as yet.

On examination of the adrenal glands of these theelin-treated animals it was found that the streaky, irregular margin of Sudanophile zone II bordering on the lipid-free zone III had disappeared and the distribution of the Sudanophile material became more like that of the other strains, forming a continuous sheet. Moreover the lipid-laden zone II had become wider and denser than the controls. Although in the adrenals of the theelin treated animals Sudanophile zone II doubled its width as compared with the controls, it was still only two-thirds that of the C57 Blacks. These facts were true for both sexes. The experiment also brought out other facts. First, the lipid-free zone was narrower than in the normal for both sexes as might be expected since the lipid or Sudanophile zone II had widened. Second, the rim of very low lipid density (zone III) measuring 1 to 2 units wide and bordering on the medulla in the normal female, had increased in width in the treated female animals. It measured from 10 to 20 units depending on where it was recorded since it was streaky and not a continuous sheet. Since this was true only for the females it indicated that it was specifically associated with the ovaries or other female endocrine conditions. The first result, affecting both male and female in the same way, does not appear to be associated with sex. Theelin, *per se*, increased the amount of lipids in the dba strain. What effect theelin has in a strain where there is already a maximum amount of lipids awaits further studies.

DISCUSSION

The work of R. Whitehead on the variations in the cortical lipid of the mouse adrenal associated with sex and age ('33) is the only study on the mouse adrenal pertinent to this work. His material, however, was not uniform. It consisted of a mixed colony of mice of unknown strains and history, and of ages from 15 to 338 days. Within this wide age limit he found, regardless of sex, three main histological types of lipid distribution. The first type found in the largest number of individuals showed a broad lipid band, occupying

not less than half the width of the permanent cortex. The second type had a narrow lipid band, less than half the width of its permanent cortex. The third type showed an irregular lipid band. He does not correlate these types with any particular age, nor with any particular physiological condition of the animal. His material coming closest to being comparable to the present material in age was his 1- to 2-month age group. He stated that at this age the "Sudanophile material was most abundant and that it occupied almost the whole width of the permanent cortex." This age group must have belonged to his first histological type. It can be also assumed that the second and third types were found chiefly among the older age groups. No variations in lipid distribution were found by him in the younger age group. The present material of 34-day-old animals showed distinct variations among the six strains. Whitehead's types were due to age differences while here the variations are due to strain differences. Since the strains here studied are of the same age but different in their mammary tumor incidences the variations of lipid distribution may well be associated with a basic physiological condition of the animal.

Such questions as these present themselves. Does the adrenal cortex loaded with lipid represent a storage phenomenon? Is there a differential need for lipids, since the relative lipid amounts range among the strains from 98% to 60% of the total cortical width? Does a greater demand for lipid by the body show a less amount present in the cortical adrenal? Miller and Riddle in a recent paper ('42) report that in the pigeon adrenal the "cortical hormones (water insoluble ketones) which are located in the lipids are quickly synthesized and excreted from the cell." They also state that in the normal gland where the demands of the animal for circulating the cortical hormone are perhaps not urgent, "a considerable storage of a precursor (cholesterol?) and of the hormone (water insoluble ketones?) may occur."

It is universally accepted that the vital secretion of the adrenal cortex is associated with lipids and that it may be

isolated from the lipid fraction obtained from the gland. The exact relation of the ketone substances to the true cortical hormones is however not yet established. Bennett ('40) demonstrated that the ketone materials can be removed with the lipid material. Miller and Riddle believe that mitochondria are in some way involved in the formation of hormones and lipids. They advance the theory that mitochondria may give rise to the lipid as rapidly as they are formed and that storage of lipid results.

Since the C57 Black, a low mammary gland tumor strain, has a high amount of visible cortical lipids it would follow from the above interpretation that this lipid would be storage lipid. On the other hand the C3H strain with a high mammary gland tumor incidence and a smaller amount of cortical lipid would demand lipid at a more rapid rate than it can be formed and so its relative visible amount has decreased. The dilute brown strain, another high mammary gland tumor incidence strain, shows still less visible lipids than the C3H strain, and its demands for lipids would on this theory evidently be greater than those of strain C3H.

The experiment of theelin injection in the dba strain suggests the possibility of an endocrine control. The administration of theelin caused an increased measurable lipid content and the X-zone to hypertrophy and to show (although in very small droplets of lipid) still a visible trace of this material. It is interesting to recall at this point that the administration of thyroid also caused the X-zone to show traces of lipid where it was lipid-free in the normal animal.

M. K. McPhail ('42) in studies of the action of hormones on the X-zone reported that oestrone may cause X-zone degeneration but is more prone to produce vacuolization of the inner fasciculata which he designates "ring of degeneration." His histological sections were prepared by routine methods. An examination of his photomicrograph does not clearly show whether the degeneration is a fatty one. Since he did not make Sudan stained sections it cannot be said that his vacuolization area and ring of degeneration contained lipid.

The theelin experiments of the present observations show first that contrary to McPhail's interpretation of a possible X-zone degeneration a definite hypertrophy of the X-zone is evident. Secondly, the inner fasciculata which is equivalent to McPhail's vacuolization area or "ring of degeneration" is lipid-free. McPhail does, however, make the statement that his results are divergent and conflicting and has no explanation for them. The majority of workers have also failed to find an X-zone destruction following oestrone administration which McPhail admits. The theelin experiment was done on one strain only. What effect would be obtained if done on the other strains can be only answered after further experimentation.

Since the X-zone of different strains varies slightly in width, the question may arise as to whether this may account for the variation in lipid amount. The width of the male X-zone is practically negligible, still there are strain differences in the widths of lipid distribution. A comparison of the absolute widths of lipid distribution not only of the male but also of the female answers this question.

As suggested by the theelin experiment, the lipid-free zone in the normal gland may very likely be made up of both the innermost fasciculata and X-zone. The latter hypertrophied and acquired small lipid droplets with theelin administration. The innermost part of the fasciculata, however, did not acquire any lipid.

The work of Woolley, Fekete and Little on the adrenal gland of the dba strain is of special note. These workers found a feminizing effect following the formation of hyperplastic adrenal nodules in castrated male mice. After ovariectomy they observed a consistent nodular hyperplasia of the adrenal cortex. They suggested that due to the close cellular similarity of these nodules to lutein-like cells of the ovaries that these hyperplastic nodules may be sources of estrogenic hormones. The lipid content of this material is of interest and will be studied with the view of correlating it with the present observations.

A further clarification of the problem of lipid control awaits additional studies on the older age groups of the various strains of mice and additional experimentation.

SUMMARY AND CONCLUSIONS

Six inbred strains of mice, three of high and three of low incidence of mammary gland tumor, were studied with regard to the cortical lipid content of the adrenal gland at age 34 days. These strains were C57 Black, N, ce, A-albino, C3H and dba or dilute brown.

The observations revealed definite information concerning the amount of adrenal cortical lipids. A high incidence of mammary gland tumor was associated with a low amount of adrenal cortical Sudanophile material or lipids. Strains with low incidence of mammary gland tumor showed relatively high lipid content. What this really means is not clear at this time.

There was no mathematically significant difference in the relative amount of lipids between the sexes of each inbred strain. The lipid-free area was, however, slightly greater in the females than in the males. This was true for all strains.

Whether the lipid-free area is equivalent only to the X-zone or to more than the X-zone region is discussed but no final conclusion can as yet be drawn.

Accessory adrenal glands were found in greater numbers in the C57 Black strain than in the other strains. They were least common in the dba strain.

The effects of theelin injections on the adrenal gland of mice (dba strain) were: (1) increased width of the Sudanophile II zone for both sexes, (2) the hypertrophy of the X-zone of the female, (3) the acquisition of very small lipid droplets by the X-zone of the female.

The question of the factors involved in the control of the amount of visible lipids in the adrenal gland is discussed. The suggestion is advanced that it may involve an interaction of certain endocrine secretions.

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AGEING OF HUMAN SKIN ¹

I. INFLUENCE OF DERMAL SHRINKAGE ON APPEARANCE OF THE EPIDERMIS IN YOUNG AND OLD FIXED TISSUES

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ONE TEXT FIGURE AND THREE PLATES (TWELVE FIGURES)

Significant advances in the formulation of basic concepts of the phenomenon of ageing must be expected, as in other fields, chiefly to come from animal experimentation. Nevertheless it is important that accurate data be available concerning the changes of senescence in human tissues. It is the purpose of this series of papers to present the results of studies of the ageing process in a carefully controlled group of human skin specimens, collected by biopsy from a single area of the body in healthy adult white males. The specimens were so handled as to eliminate as many variables as might be possible in human material, and an effort was made to employ a variety of histological and physico-chemical techniques. It is hoped that correlations ultimately will develop such that an integrated picture can be visualized of the processes of ageing in this single tissue.

The studies reported in this first paper were designed to clarify the rather nebulous term, "senile atrophy" of the skin. Many textbooks of dermatology refer to the thin skin of old age, but whether this thinning implies a change in the epidermis, in the dermis, or in both rarely is clear. Macleod (40)

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² Now Captain, M. C. AUS. Office of the Surgeon General, Washington

states that the epidermis is thinner in old age but no evidence is given. Ejiri ('37) counted the number of layers of epidermal cells in young and old cadavers. He states that with advancing age the number of layers diminishes on the head and face and increases on the dorsal surfaces of the arm, hand and foot. He disagrees with the statement of Saalfeld that the number of granular layers decreases with advancing age. (It has not been possible to locate the paper by Saalfeld wherein this statement is made). Ejiri refers to atrophy of rete ridges in old skin as do Hill and Montgomery ('40). Weidman ('42) states that there is no doubt that the epidermis undergoes atrophy with advancing age, but atrophy is not clearly defined.

Ceresa ('37) studied the skin from different regions in individuals ranging in age from the fifth month to the ninety-third year. Measurements of the thickness of the dermis are given, but the epidermis was not studied. Ceresa also counted the number of dermal papillae per linear unit in skin sections, reporting a moderate diminution in old age. Becker ('21) in a study limited to infants found a tendency for the skin over the biceps and triceps and over the radius to be thinner at 6 months of age than at birth. Atsuki ('34) studied skin thickness in newborn and adult individuals. Difficulties were experienced in having this article translated; apparently the measurements were made grossly by means of calipers ("a metal instrument"). Atsuki concluded that skin thickness changes in relation to many variables, of which age is one; adults, in general, had thicker skins than infants.

None of the papers cited gives a clear definition of the term, atrophy, especially as regards microscopic changes in the epidermis. The gross changes are sufficiently obvious to require but little description, and it might seem, on causal inspection, that microscopic differences between young and old epidermis are equally obvious (figs. 2 and 4). The young epidermis seems markedly thicker, and massive by contrast; its ridges are more robust and some may project farther into the dermis. However the young skin also is more folded, more distorted than the old, and the possibility should be considered

that its massiveness might be explained by a more vigorous shrinkage of the connective tissue, this shrinkage pressing together the epidermal cells from side to side and drawing the ridges downward. The difference in shrinkage is apparent at biopsy: young skins retract vigorously immediately after removal but old ones remain relatively smooth and flat.

In order to test the assumption that dermal responses to fixation largely determine the typical picture of the epidermis presented by a histological section, a series of models of epidermis was constructed as detailed in a later portion of this paper. These models included reconstructions of epidermis fixed with dermal connections intact (usual method of fixation of skin), and reconstructions of epidermis separated from the dermis just after it was removed by biopsy and subsequently fixed.

It was believed that the most direct approach to the problem was a determination of the total volume of epidermis present in a unit of surface area of the skin. Such determinations were done by the area-weight method on three young and three old specimens and a roughly quantitative estimate of shrinkage was made of an area of skin outlined in India ink and measured before biopsy, immediately after biopsy and before fixation, after fixation, and during dehydration.

MATERIAL, METHODS AND OBSERVATIONS

It was believed that a relatively complete body of data concerning a single area would be more useful than the more diffuse information to be obtained from a regional survey, and that this area should be so located that observations would be unaffected, as far as possible, by environmental influences. The antecubital region seemed best to meet the requirement for the following reasons: it is protected by its position from many radiant and mechanical traumata even when uncovered; clothing usually is loose over this region and rubbing is at a minimum; sufficient "slack" is provided partially to compensate for the pulling and stretching of movement; the relative

efficiency of evaporation and the absence of apocrine glands minimize a possible physico-chemical interference.

All specimens were obtained by biopsy from patients in two institutions for indigent or mentally deficient cases, and in each instance clinical records were studied in order to eliminate subjects whose physical condition might have introduced a confusing factor. Because of the known tendency to greater individual variation in females, only male subjects were used. A similar tendency toward greater individual variation in very young organisms suggested limitation of the study to adults; the young individuals, therefore, were selected from an age group of 19 to 30 years. Technical difficulties inherent in many of the methods required that they be restricted to surveys of the extreme limits of the adult range; in such surveys the old individuals were 80 years old or more. When simpler techniques were used, a middle-aged group also was included.

The technique of biopsy was standardized as follows: the field was washed gently with bland soap and water, and was rinsed with 70% alcohol. Blebs were raised in the skin by intracutaneous injection of a solution of 2% novocaine in physiological saline, so as to produce a continuous ridge surrounding an oval area of several square centimeters, varying in extent according to the amount of tissue required. None of the anaesthetic was injected subcutaneously and the tissue immediately was removed by an elliptical incision within the anaesthetized area, so placed that its edge was everywhere at least 0.5 cm. from the novocaine blebs. This precaution seemed advisable in order to minimize possible volume changes and chemical changes resulting from altered osmotic conditions. After division into the required number of pieces, the tissue was straightened gently on paper and immediately was placed in the fixative or otherwise stored for physico-chemical studies.

Precautions were taken to ensure uniformity of shrinkage. All specimens were fixed in Bouin's fluid for 24 hours and the methods of dehydration, of imbedding in paraffin, of sectioning

and of staining were standardized. Harris' modification of Delafield's haematoxylin with eosin as counterstain was used routinely on all tissues. In addition, special fixatives and special staining methods were employed in a number of studies to be reported in subsequent papers. The tissues used for this first study were fixed in Bouin's fluid and were stained with haematoxylin and eosin.

Serial reconstructions. Serial sections, 10 micra thick of two specimens (A-22 and A-28), 20 and 86 years old, respectively, were cut and stained with the precautions just outlined. Camera lucida tracings of the epidermis, excluding the corneum, were made at a magnification of 160 diameters on blotting paper of uniform thickness. The resulting figures were cut out and glued together in their proper order to make the models. The corneum was not drawn because in many areas it was distorted, and in some it was separated entirely from the stratum lucidum.

It was planned to construct models of specimens from the same individuals, using epidermis separated from the dermis before fixation, in order to demonstrate the effect of dermal shrinkage on the appearance of the epidermis. However, more tissue was required and the 86-year-old A-28 would not permit another biopsy. A second specimen was obtained from the 20-year-old A-22, and a specimen from an 89-year-old subject (A-30) was substituted for A-28.

The separation of epidermis from dermis was carried out by Dr. V. Suntzeff, using the method of dry heat at 50°C., recently developed in the laboratories of the Barnard Hospital (Baumberger, Cowdry and Suntzeff, '42). The elevated temperature loosens the epidermis in about 2 minutes and it can be lifted intact from the dermis. The separated epidermis was floated on the surface of a solution of physiological saline, and curled edges were straightened gently with teasing needles. The flattened epidermis then was transferred to Bouin's fluid, imbedded, sectioned serially at 5 micra, and stained. The naked pieces of dermis were sectioned and examined to insure that no portions of epidermis had remained

in the areas drawn. Models of the separated epidermises were constructed as before.

The models of epidermis fixed with dermal connections intact (figs. 6 to 9) show the same marked differences noted in the stained sections (figs. 2 and 4); the relative massiveness of the young specimen is even more striking in the three dimensional figure. However, the distortion and folding of the young and the relative flatness of the old also are apparent. The surface folds of the young specimen are drawn so close together and are so deep that it is difficult to follow the pattern of the rete ridges on the dermal surface. By contrast, the ridge pattern is clear in the old specimen. The appearance is suggestive of a vigorous shrinkage of connective tissue in the young specimen such that the contrast after fixation is misleading: it seems not improbable that *in vivo* the young epidermis, in thickness and conformity, is not greatly different from the old; that its massive appearance in stained sections is to a large extent illusory, and is chiefly the result of connective tissue shrinkage which results in a crowding together of the cells of the epidermis.

The models of separated epidermis (figs. 10 to 13) tend to support this impression as do the single stained sections (figs. 3 and 5); much of the marked difference in thickness and the relative bulkiness of the young specimen have disappeared. Indeed, both specimens are so flattened that almost the entire ridge pattern has disappeared also, and it could follow that, without the dermal "tone", both specimens would present, *in vivo*, a smooth undersurface. However, these models should be interpreted with caution. The tissues were handled as gently as possible, but the heat and the stretching incident to straightening may have influenced the result so that the young specimen looks deceptively thin. Nevertheless, it is believed that they lend support to the theory that the appearance of the epidermis in stained sections, and especially the marked differences between young and old specimens, are, to a large extent dependent upon dermal influences.

Shrinkage and volume. For this study, an area with dimensions of 1.0 by 0.5 cm. or 0.5 by 0.5 cm. was measured with a micrometer caliper on the skin of the antecubital region. The limits of the area were drawn in India ink with a fine pen before biopsy, the arm being fully extended. Full extension, without further manipulation of the skin by the observer eliminated all gross folding, gave sufficient tautness for inking in the margins, and served as a rough control to ensure that the area enclosed approximately the same amount of skin in each subject. A margin of skin several millimeters wide was left around the inked line during fixation and dehydration; this margin was cut away with a sharp razor just before infiltration with paraffin. The dimensions of the area were measured by micrometer caliper after biopsy and before fixation, after fixation, and after dehydration.

The entire block was sectioned serially at 10 micra, and the sections were stained in the usual manner and were counted. The outlines of the epidermis, excluding the corneum, of a representative sample of the total number of sections were drawn by camera lucida at a magnification of 91 diameters. These outlines were transferred to no. 3 Kodaloid and were cut out and weighed in accordance with the method described by Scammon and Scott ('27). Knowing the weight of 1 sq. cm. of Kodaloid and the magnification used, it was possible by simple proportion to determine the area of the epidermis in the sections drawn. Multiplication by the section thickness of 10 micra gave the volume of epidermis in the sample; this was recorded as cubic millimeters of epidermis per square centimeter of skin surface by relating it to the total number of sections and to the dimensions of the area drawn on the skin.

In order to be certain that the sections drawn were representative of the entire block, one specimen was handled such that the Kodaloid weight was determined which represented 2.5%, 5%, 7.5%, and 10% of the block. The average figure in each of the three repeated samples was within 1% of the figure representing the average of the preceding series. The remaining specimens, therefore, were drawn in similar groups, and the

sample was considered representative when the average weight of the total number drawn agreed, within 1%, with the average computed for the series ending with the preceding 2.5% of the total number of sections.

As was expected from gross observation, the difference in shrinkage between young and old was marked (table 1). The young skins lost from 38% to 58% of their original areas; each of two old skins lost 12%, and in one instance the area actually increased by 20% after removal. Figure 1 shows these results diagrammatically and indicates somewhat the degree of "crowding" to which the epidermal cells are subjected in the young specimens.

TABLE 1
Shrinkage and volume studies in young and old skin.

SPECIMEN	AGE	AREA BEFORE BIOPSY	AREA AFTER FIXATION	SHRINKAGE	VOLUME EPIDERMIS
		(sq. cm.)	(sq. cm.)		(cu. mm. per sq. cm.)
A-32	22	0.50	0.31	38%	3.67
A-35	24	0.50	0.21	58%	3.10
A-36	32	0.25	0.14	44%	3.58
A-34	89½	0.50	0.44	12%	3.02
A-37	93	0.25	0.30	+20%	3.64
A-38	81½	0.25	0.22	12%	3.05

The volume studies showed a wide individual variation (table 1). The method of laying out the original area on the skin, however, could never be extremely precise because of variations in the width of the inked line and in the degree of tautness of the skin; it is estimated that the total error could be as high as 5%, including the error in drawing, cutting and weighing of about 1%. The figures, therefore, cannot fairly be assumed to indicate the absolute volume of epidermis present, and do not admit of statistical treatment. They do, nevertheless, clearly support the original premise: if the difference between young and old epidermis were actually as marked as it appears to be in stained sections, the volumes as determined would show an unequivocal difference.

It was believed desirable to add to this series, and ten additional specimens were collected, five young and five old. It also seemed worth while to determine how great a rôle was played by differences between young and old in loss of epidermal volume during fixation. It is generally agreed that the smallest loss occurs in tissues fixed by freezing in isopentane and liquid oxygen with subsequent dehydration in vacuo.

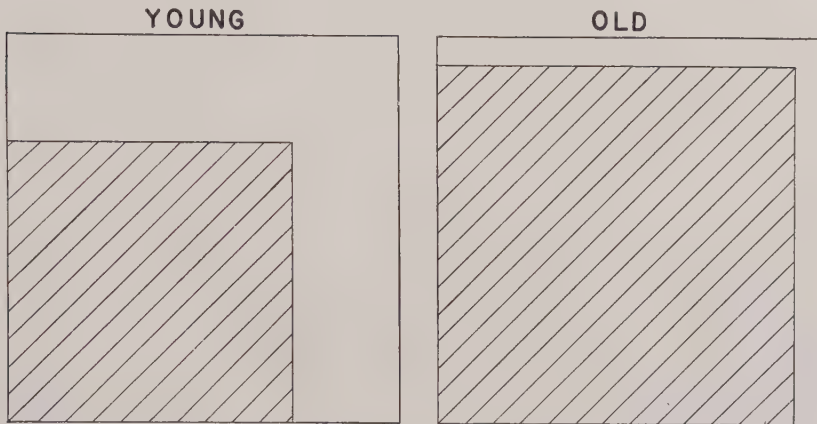


Fig. 1 Diagram illustrating differences in shrinkage of typical young and old skins. Shaded portions represent the areas after fixation which occupied the entire square before biopsy. The diagrams are drawn as though shrinkage occurred equally in all directions. The percent of shrinkage used for these diagrams represents the average of the figures given in table 1, except that the figure for A-37 (a gain in area of 20%) has been omitted.

Half of the marked area in each of six of the new specimens, therefore, was fixed by this method and the other half was fixed in Bouin's fluid; it was planned to study volume differences in the two types of fixation, assuming the frozen-dehydrated specimen to represent the nearest approach to conditions in vivo. Unfortunately the method of volume determination is time-consuming, and changed conditions in this laboratory due to the war have made it necessary to postpone this work. It is hoped that it can be presented later.

Epidermal thickness; the number of cell layers. The observations just discussed are believed to demonstrate the correct-

ness of the theory that without the large difference in the reaction of their connective tissue beds, young and old epidermises would show very little difference in thickness or conformity. It seemed desirable, however, to have on record some data concerning thickness of the epidermis and the number of layers of cells present in stained sections. The distortion in fixed sections of young skin and the difficulty of deciding whether a section through the epidermis has passed obliquely or perpendicularly through a given ridge make quantitative measurements of ridge thickness too doubtful to be of value. The same criticism applies to counts of the number of cell layers in these areas. Accordingly, quantitative methods were applied only to the areas between rete ridges where errors of measurement due to the angle of cutting could be avoided with greater precision. Even so, the figures do not, of course, represent true thicknesses; the folding and distortion, and the crowding together of the mass of cells by dermal shrinkage must be taken into consideration.

Specimens from fourteen subjects, seven young and seven old, were collected and prepared with the usual precautions, sections being cut serially at 5 micra. Measurements of epidermal thickness in the areas between rete ridges were made by ocular micrometer at a magnification of 1425 diameters. Exclusion of obliquely sectioned areas was necessary; a given area was accepted as a true cross section when the basal cells appeared columnar, with gradual and uniform flattening in the distal layers. In order to cover a wide field, readings were taken in order from one end of the section to the other, a distance of about 0.5 cm. When this length did not yield the required number of readings as discussed below, measurements were continued on a section in the series 50 micra distant from the first, in order to exclude immediately adjacent areas.

Measurements were made by two observers. Alternating readings, one observer selected an inter-ridge area believed to be a true cross section, adjusted the micrometer and recorded the thickness from the proximal pole of the basal nucleus to the distal pole of the outermost granular nucleus.

The reading was checked by the other observer, who then adjusted the micrometer and selected the area for the next reading. Mean values were computed at the completion of every tenth reading and the recorded mean was considered representative of the skin under observation when the mean value of the total number of readings agreed, within 0.03 ocular division with the mean value recorded ten readings previously. Readings were taken to the nearest 0.05 division (1.00 division was equivalent to 10 micra), and at least twenty readings were made for each specimen. The total number of cell layers and the number of granular layers were recorded separately for each area in which the thickness was measured.

TABLE 2
Epidermal thickness.

SPEC- IMEN	AGE	NO. OF READ- INGS	THICK- NESS	CELL LAYERS	CELL LAYERS	SPEC- IMEN	AGE	NO. OF READ- INGS	THICK- NESS	CELL LAYERS	CELL LAYERS
			(mi- cra)	(Total)	(Gran- ular)				(mi- cra)	(Total)	(Gran- ular)
A-8	19	30	36.1	6.17	1.50	A-13	80	30	27.6	5.30	0.97
A-15	30	30	33.5	6.20	1.83	A-14	85	20	28.3	6.10	1.00
A-21	20	20	35.1	5.90	2.00	A-16	91	20	27.5	5.00	1.00
A-22	20	40	35.4	6.17	1.92	A-26	94	20	31.6	5.60	1.25
A-23	24	30	32.9	5.80	1.46	A-27	88	20	27.0	5.10	0.90
A-24	24	40	34.3	6.30	1.87	A-28	86	20	23.2	5.10	1.00
A-25	20	40	29.5	5.40	1.13	A-29	86	30	26.2	5.30	1.00
Means young group			33.8	5.99	1.67	Means old group			27.3	5.36	1.02

The seven young skins were thicker than the old by an average of 6.5 micra (table 2) and showed a tendency to be provided with a layer of cells in the stratum granulosum not present in the old. (Differences between means of 0.63 layer in the total count, 0.65 layer in the granular count). The results were analysed by the small sample technique of "Student" as developed by Fisher ('28), employing the value, *t*. The simplified formula of Rider ('39) was used to compute the estimated standard deviation of the difference between means and was used as *s'* in the formula for determining *t*. The results are given in table 3.

From a statistical point of view, therefore, it is highly unlikely that chance could account for the differences shown. This statement, however, should be interpreted in the light of the results obtained in the studies on volume and shrinkage. It seems probable that the tendency toward an extra layer of granular cells in the young specimen represents a real difference and that the difference in thickness of 6.5 micra is real insofar as it applies to the dimensions of this layer of cells. The remainder of the 6.5 micra may be attributable possibly to the effect of greater shrinkage in the young specimen, as discussed.

TABLE 3
Analysis of data: epidermal thickness.

	DIFFERENCE BETWEEN MEANS ($\bar{x}_1 - \bar{x}_2$)	s'	t	P
Thickness Epidermis	6.5 micra	0.2361	5.1469	.01
Cell layers (total)	0.63 layer	0.3493	3.3422	.01
Cell layers (granular)	0.65 layer	0.2368	5.0821	.01

s' indicates the estimated standard deviation of the difference between means as determined by the formula of Rider; P , from Fisher's table IV, represents the probability for values of t recorded.

The tendency to lose a layer of granular cells in old specimens suggested measurements of the corneum, but it was poorly preserved in most skins, and a sufficient number of areas with a perpendicular plane of section could not be found. However an impression of greater thickness in young corneums had been obtained from observation of this series, as well as of many other epidermises from the same area.

DISCUSSION

The term, senile atrophy of the skin, is not definitive. Especially is this true with regard to the epidermis, since a proved alteration in the connective tissue need imply no change at all in the covering layers. It is important, there-

fore, sharply to discriminate between epidermis, dermis and subcutaneous tissue in using such terms as thinness and atrophy.

As MacCallum ('41) points out, the word atrophy itself may imply many concepts. In the anatomical sense, one usually applies it to a tissue which has decreased in bulk through "wasting" of its component elements whether they are living cells or inert supporting substances. But even in this restricted sense there is opportunity for confusion. An epithelial tissue might decrease in bulk through reduction in number of its cells with no change at all in the size of the cells themselves; or the reverse might be true. In the first instance, a decreased rate of cell production or an increased rate of cell death might be responsible; in the second, neither of these factors need be operable, but both conceivably might be, together with the change in individual cell size. Furthermore the term atrophy apparently is broad enough to include functional alterations, whether or not such alterations are accompanied by changes visible anatomically. Confusion of the independent with the interdependent factors can lead to unwarranted generalizations.

It seemed most useful to begin studies of the effects of senility in the skin with a detailed inquiry into the question of how far the word atrophy could be applied to the senile epidermis in the restricted anatomical sense. It is improbable that the total volume of epidermis is markedly decreased in the area studied in old skins, for volume determinations in six specimens did not show any significant difference between young and old. It is interesting in this connection to reflect that an adult male, 170 cm. tall and weighing 70 kg., with body surface area of 1.77 square meters as calculated by the DuBois formula, would possess, according to the results discussed, a total volume of epidermis varying from about 54 cu. cm. to about 65 cu. cm., excluding the corneum. (This estimate, of course, disregards the well known regional variation in thickness.)

With regard to the type of atrophy which results from alterations in the normal rhythm of cell division and cell death,

it is not improbable that this change does occur in the epidermis; old skins tend to lose a layer of granular cells. Furthermore the corneum and the basal-granular dimension of the epidermis tend to be thinner in old age. These results, of course, were obtained by methods which necessitated considerable subjective manipulation, and the effect on them of dermal shrinkage must have been great. Very little definite information is available concerning the stability of a sheet of epidermal cells, although the prevailing view is that the intercellular bridges produce a rigid structure. If the cells were able freely to glide over one another, the absence of the layer of granular cells in old skins could be explained simply by a piling up of cells in young specimens because of more vigorous dermal shrinkage. This would be unlikely, however, without a similar increase in the number of layers in the lower strata, which was never found. It seems more probable that the loss of a granular layer and possibly a thinner corneum represent a true decrease in bulk. But these are minute changes, detectable only after careful examination. They do not fit in with the striking difference between young and old apparent in stained sections. There is little doubt that most of this difference is the result of greater shrinkage of the young connective tissue.

The basal cells of old epidermis tend to be rounder in cross section, less columnar than those of young epidermis. It is probable that this change, together with similar alterations in the upper strata, is again the result of differences in dermal tension. It seemed desirable to extend this study of the anatomical manifestations of atrophy to a consideration of individual epidermal cells. A later paper of the series will report studies of cell and nuclear volumes and nucleo-cytoplasmic ratios in young and old epidermis.

SUMMARY AND CONCLUSIONS

1. Measurements of epidermal thickness from basal to granular layers in the areas between rete ridges showed old epidermis to be thinner than young by 6.5 micra. Old skins

also had a tendency to lose a layer of granular cells and their corneums appeared to be thinner.

2. These changes are too minute to explain the striking differences between young and old seen in stained sections. They may be partly illusory due to distortion and crowding of the mass of cells by the greater shrinkage of connective tissue in young specimens. This idea is supported by volume studies in six specimens in which a significant diminution in old age of total epidermal volume per square centimeter of skin surface could not be demonstrated. That the dermal shrinkage is decidedly more vigorous in young specimens was shown by measurement before removal and after fixation of marked areas on the skin. The theory is further supported by comparison of serial reconstructions of young and old epidermis fixed with dermal connections intact and after separation from the dermis. Removal of the dermis abolished much of the marked difference between young and old.

3. It is probable that, except for the marked difference in reaction of their connective tissue beds, young and old epidermis would show very little difference in thickness or conformity.

We are indebted to Mr. J. G. Steinle and to the late Dr. C. A. Bauer of the St. Louis City Infirmary, to Drs. A. A. Heinz and S. S. Nemas of the St. Louis Training School, and to the patients in these institutions who cooperated so willingly throughout the series of studies.

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PLATE 1

EXPLANATION OF FIGURES

2 Photomicrograph of a section of skin fixed with dermal connections intact. Skin taken by biopsy from the anterior cubital region of a male aged 20 years (A-22), 10 μ , $\times$ 190.

3 Photomicrograph of a section of epidermis separated from the dermis before fixation. Same individual and same region as in figure 1. 5 μ , $\times$ 190.

4 Photomicrograph of a section of skin, fixed with dermal connections intact. Skin taken by biopsy from the anterior cubital region of a male aged 86 years. (A-28) 10 μ , $\times$ 190.

5 Photomicrograph of a section of epidermis separated from the dermis before fixation; from a male aged 89 (A-30). 5 μ , $\times$ 190.

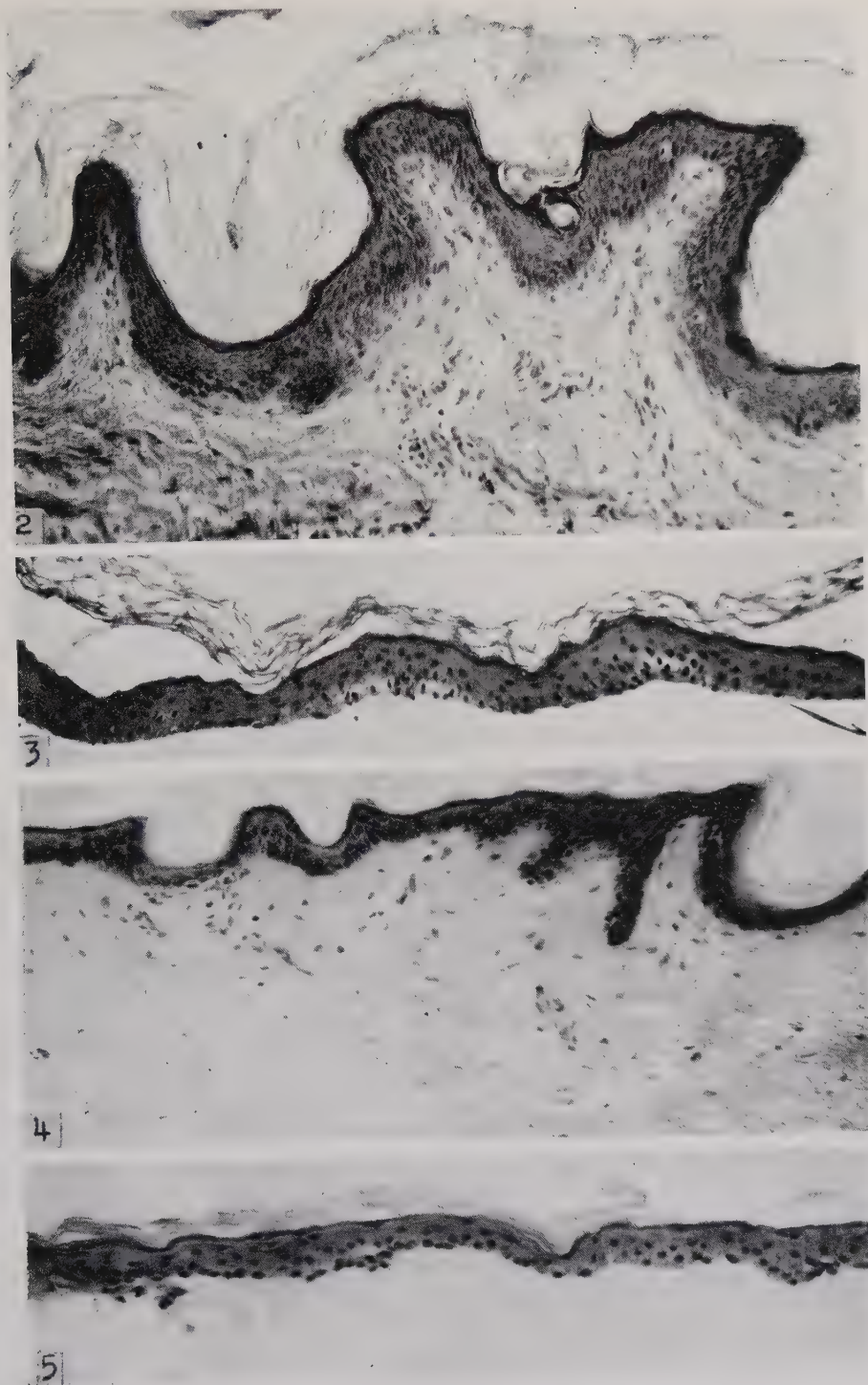


PLATE 2

EXPLANATION OF FIGURES

Blotting paper models of epidermis taken by biopsy from the anterior cubital region of male subjects. The epidermis was fixed with dermal connections intact. The orifices of ducts of sweat glands are indicated by d; hair follicles are labeled h. $\times 60$.

- 6 Free surface of the epidermis from a 20-year-old specimen (A-22).
- 7 Dermal surface of same.
- 8 Free surface of epidermis from an 86-year-old specimen (A-28).
- 9 Dermal surface of same.

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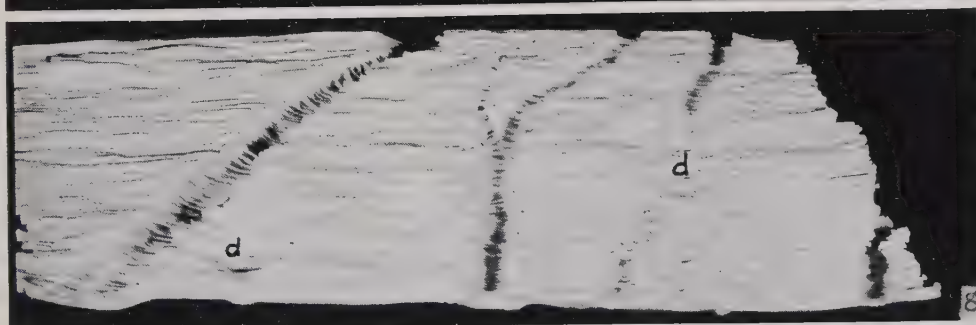
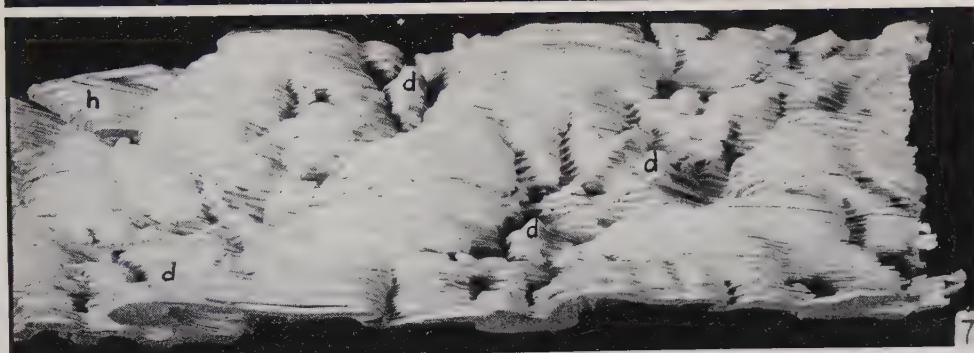
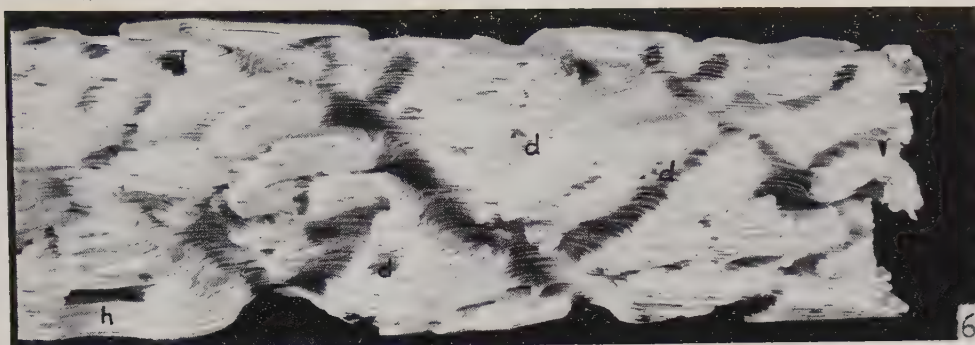


PLATE 3

EXPLANATION OF FIGURES

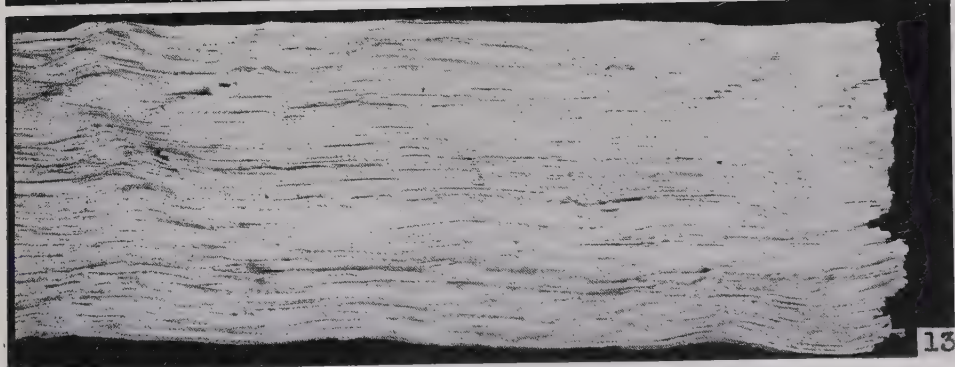
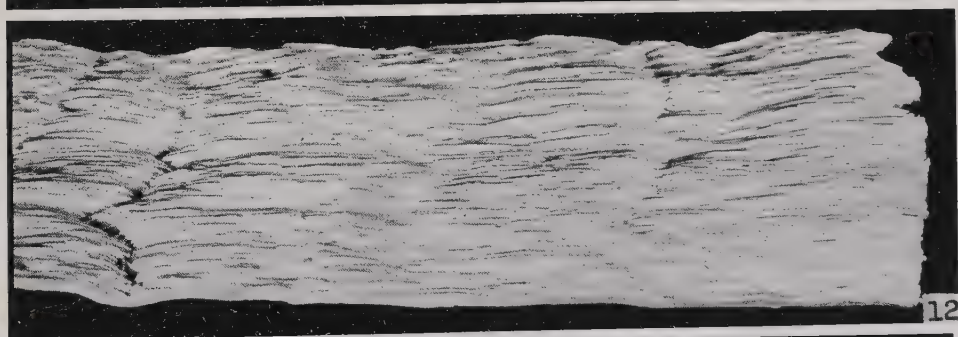
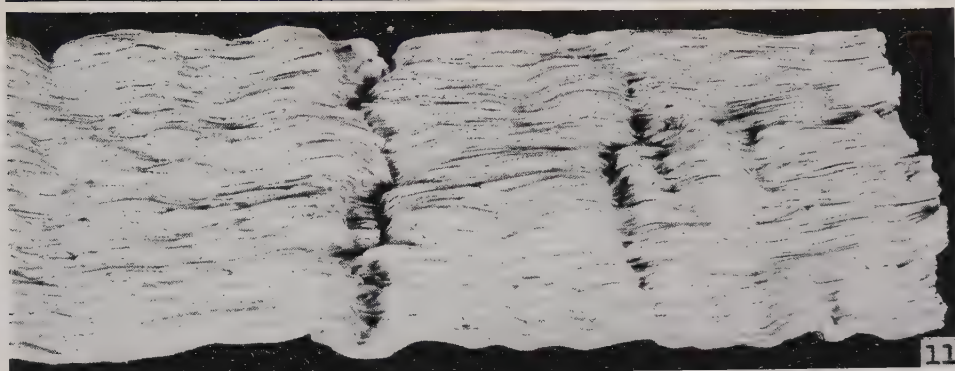
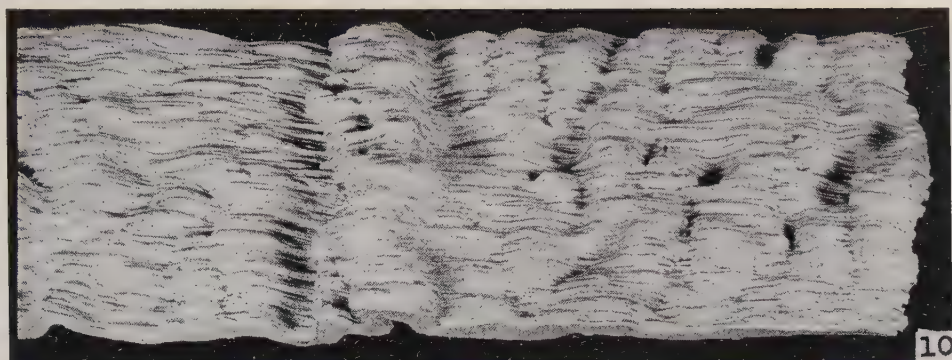
Photographs of blotting paper models of epidermis taken by biopsy from the anterior cubital region of male subjects. The epidermis was separated from the dermis by heat (as referred to in the text) and subsequently fixed. Orifices of ducts of sweat glands are indicated by d; hair follicles are labeled h. $\times 60$.

10 Free surface of the epidermis from a 20-year-old subject (A-22) (fig. 3). The large fold is an artifact.

11 Dermal surface of same.

12 Free surface of epidermis from an 89-year-old specimen (A-30). (See also fig. 5).

13 Dermal surface of same.



REPRODUCTION IN THE CHIMPANZEE: REPORT ON FORTY-NINE BIRTHS¹

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Yerkes and Elder ('37, p. 43) have reported twenty chimpanzee births which occurred in these laboratories prior to July 1, 1937. The present note supplements these earlier observations on the basis of twenty-nine additional cases which have ensued since that time and up to April 1, 1943. There being one twin birth, the record covers a total of 50 infants.

GESTATIONAL LENGTH AND VARIABILITY

Table 1 lists all forty-nine cases, including the twenty reported by Yerkes and Elder, in order of successive pregnancies for each female. The length of gestation as recorded obviously depends on our estimate of the date of conception. It should be noted that the latter is known by the procedure of single matings in cases 13, 18, 19, 21, 25, and 37, and is estimated in all other instances. The first column of gestational periods shown in table 1 is based on the original estimates as recorded in the laboratory files. Omitting case 26, for which the data are incomplete, the average for the remaining forty-eight cases is 228.7 days with a standard deviation of 12.7 days. The median is 228.5 days; the range is from 202 to 261 days.

In making the original estimates of conception dates, especially the earlier ones, the mid-point of the cycle was given prominence as a criterion of the time of ovulation (Elder and Yerkes, '36, p. 141; Yerkes and Elder, '36, p. 278). Although

¹ Acknowledgment is made to the Committee for Research in Problems of Sex, National Research Council, for partial support of the observational work which is basic to this report.

TABLE 1

Chimpanzee births to April 1, 1943. Listed in order of successive births for each female, the latter appearing in alphabetical order

CASE ¹	MOTHER	MOTHER'S AGE IN YEARS AT PARTURITION ²	ESTIMATED GESTATIONAL PERIOD IN DAYS ³		ORDINAL OF PREGNANCY ⁴	DATE OF BIRTH	INFANT	FATHER
			Orig.	Rev.				
1	<i>Alpha</i>	9	235	235	1	10.17.39	Alf	Frank
2	<i>Alpha</i>	10	220	220	2	1.28.41	Flora	Frank
3	<i>Alpha</i>	12	227	227	3	4.29.42	Falla	Frank
4	<i>Bentia</i>	14	224	224	1	6.21.40	Stib	Jack
5	<i>Bentia</i>	16	238	236	2	4. 7.42	Jent	Jack
6	<i>Bimba</i>	11	242	241	1 (2)	1. 2.41	Banka	Frank
7	<i>Bimba</i>	13	237	237	2 (3)	6.15.42	Brinka	Frank
8	<i>Bula</i>	12	236	236	1	5.26.42	Karla	Frank
9	<i>Cuba</i>	9	245	243	1	4.11.35	Peter	Bokar
10	<i>Cuba</i>	12	223	223	2	12.15.37	Kola	Jack
11	<i>Cuba</i>	16	207	207	3 (5)	7.31.42	Cub	Bokar
12	<i>Dita</i>	14	261	260	2 (3)	4.21.34	Rosy	Jack
13	<i>Dita</i>	17	240	240	3 (4)	4.13.37	Cap	Bokar
14	<i>Dita</i>	20	207	207	4 (5)	2.22.40	Bard	Bokar
15	<i>Dwina</i>	9	246	242	1	9.11.30	Alpha	Pan
16	<i>Fifi</i>	13	216	216	2	10.31.31	Beta	Jack
17	<i>Fifi</i>	15	216	212	3	11.21.33	Delta	Jack
18	<i>Fifi</i>	19	203	203	4 (5)	5. 8.37	Jule	Bokar
19	<i>Fifi</i>	21	227	227	5 (6)	7.20.39	Art	Bokar
20	<i>Josie</i>	11	243	231	1 (2)	7.15.33	Dick	Bill
21	<i>Josie</i>	13	233	233	2 (3)	10 .7.35	Hal	Jack
22	<i>Josie</i>	18	221	222	3 (4)	4.23.40	Jojo	Jack
23	<i>Josie</i>	19	218	216	4 (5)	5.16.41	Mars	Frank
24	<i>May</i>	11	224	224	1	1.27.36	Lu	Jack
25	<i>May</i>	12	228	228	2	11.10.37	Fin	Bokar
26	<i>Mona</i>	18	<257	<257	3	10.27.31	Mon	Jack
27	<i>Mona</i>	20	216	206	4 (5)	6.26.33	(Tom (Helene	Pan
28	<i>Mona</i>	23	239	239	5 (7)	6.26.36	Ami	Jack
29	<i>Mona</i>	26	246	246	6 (8)	4.12.39	Mu	Jack
30	<i>Nana</i>	10	221	217	1	9.21.32	Gamma	Bill
31	<i>Nana</i>	11	235	230	2	5. 2.34	Don	Pan
32	<i>Nana</i>	15	226	226	3 (4 or 5)	1.19.38	Dan	Jack
33	<i>Nana</i>	17	234	234	4 (5 or 6)	2.11.40	Bona	Bokar
34	<i>Nana</i>	19	234	234	5 (6 or 7)	8.16.41	Fanny	Frank
35	<i>Nana</i>	20	216	216	6 (7 or 8)	3. 5.43	Fauna	Frank
36	<i>Pati</i>	13	248	248	2	5.14.33	Ben	Jack
37	<i>Pati</i>	17	237	237	3 (5)	3.27.37	Dina	Bokar
38	<i>Pati</i>	19	229	228	4 (6)	3. 9.39	Gar	Bokar
39	<i>Pati</i>	20	233	232	5 (7)	3. 6.40	Japa	Jack
40	<i>Soda</i>	10	236	235	1	5. 5.37	Coma	Bokar
41	<i>Soda</i>	12	232	232	2	7. 9.39	Ken	Frank
42	<i>Soda</i>	13	231	230	3	11.24.40	Scarf	Frank
43	<i>Soda</i>	15	228	228	4	5.22.42	Spratt	Jack
44	<i>Wendy</i>	10	225	222	1	6.27.33	Bob	Bill
45	<i>Wendy</i>	13	202	202	2	1.16.36	Tim	Bokar
46	<i>Wendy</i>	15	244	241	3	1.18.38	Eve	Bokar
47	<i>Wendy</i>	17	220	220	4	2.24.40	Jenny	Jack
48	<i>Wendy</i>	18	223	222	5	4.16.41	Jed	Jack
49	<i>Wendy</i>	20	204	204	6	1.16.43	Web	Bokar

¹ The twenty-nine new and previously unreported cases are italicized.

² To the nearest year. Ages are estimated except for Alpha, Bula, and Cuba whose birth dates are known.

³ Gestation is calculated from the estimated date of conception to the date of parturition. (The former date is excluded, the latter one is included in these figures.) Left-hand column (Orig.) based on original estimates, right-hand column (Rev.) on revised estimates; see text.

⁴ Excluding miscarriages. Figures in parentheses indicate ordinal of pregnancies including miscarriages.

the probable relationship of genital swelling to ovulation was explicitly recognized (Elder, '38, p. 351) it was not used as the primary criterion of ovulation time in the early laboratory records. Subsequent observations (Young and Yerkes, '43), which have included operative procedures, indicate that the termination of tumescence (i.e. the beginning of relaxation of the sexual skin swelling) — which may or may not coincide with the mid-point of the cycle — is a more reliable and exact index of ovulation in fully mature females. From these studies it appears that ovulation precedes detumescence by a day or two, probably never by more than 5 days. In the light of this newer information, the records of all forty-nine cases were re-examined, in consultation with Doctor Young, and some of the estimates of conception date and the corresponding gestation lengths were accordingly revised. Whereas the original estimates placed conception an average of 3.9 days before the beginning of detumescence, with a range of from 1 to 15 days, the revised estimates give a comparable average of 2.7 days with a range of from 1 to 5 days. The average gestational length for forty-eight cases (case 26 again being omitted) according to these revised estimates, is 227.5 days, standard deviation 12.6 days, median 228 days, range 202 to 260 days.

Case 12, with a period of 260 days, stands well apart from the others, the next longest gestational length being 248 days. It is just possible that one menstruation was overlooked and that this animal became pregnant one cycle later; this would make the gestational period only 226 days long. The group figures would then become: average 226.8, S.D. 11.7, median 228, and range 202 to 248 days.

Inasmuch as neither extreme falls more than 2.6 S.D.'s from the mean in any one of the above calculations, there seems to be no statistical justification for omitting any of the forty-eight measures in calculating the measure of central tendency. On the other hand, it does seem probable that unusual or abnormal conditions such as illness, extremes of climate, and excessive emotional stimulation would more often shorten than lengthen the term of pregnancy. Most of the pregnancies

shorter than 220 days were associated with more or less obvious signs of abnormality. For perfectly healthy animals, maintained under ideal conditions, therefore, the average duration of gestation would doubtless be longer than that indicated above.

The revised estimates of date of conception fall on the twelfth to thirty-second cycle day (forty-seven cases), the first day of the preceding menstruation being considered the first cycle day. This range of the fertile period is somewhat wider than that previously reported (Elder, '38, p. 362). The median number of days from onset of menstruation to and including the estimated day of conception is 19; the average is 21.0 days with an S.D. of 4.4 days. The resulting average menstrual age at parturition of 248.5 days (227.5 plus 21.0) for chimpanzee may be compared with the generally accepted figure for human pregnancy of about 280 days (Hotelling and Hotelling, '32, p. 656; Schultz and Snyder, '35, p. 199). For the macaque, Schultz and Snyder ('35, p. 199) give the menstrual age as 178 days, based on Hartman's ('32) determination of 163 days as conception age.

It appears that some females, such as Mona, Pati and Bimba, typically have longer gestations than others, such as Fifi. It may be noted that the one short pregnancy occurring among the former group (case 27) is associated with twinning. Nevertheless, the variability among the several pregnancies of each individual (average A.D. = 7.8 days for thirteen chimpanzees having more than one pregnancy) is somewhat greater than the variability among the averages for these same thirteen individuals (A.D. = 5.2 days). This is contrary to the finding of Hotelling and Hotelling ('32, p. 656) who, in reference to their analysis of human data, report that "... the differences between the average pregnancy duration of individuals are significantly greater than the differences between the various pregnancies of each individual." In his survey of pregnancy duration in various species, Snyder ('38, p. 594) says, "Between various pregnancies of one individual, there may be less difference in duration than between the average

durations of pregnancies of various individuals within the same race." It seems possible that the discrepancy is a function of the relatively small number of chimpanzee pregnancies on record.

CORRELATIONS WITH GESTATIONAL LENGTH

The seven infants born after a term of 207 days or less were considerably lighter than average. The rank-order correlation between length of gestation and birth weight in twenty-three cases for which length of gestation and birth weight are known is $+0.486$ with a P.E. of $.110$. Stib (case 4) was dead when found approximately half an hour after birth. He probably died during or shortly after parturition. All other infants were apparently normal at birth.

There is a tendency for early pregnancies to be longer than later ones. The rank order of length of gestation was determined for each chimpanzee mother having had two or more pregnancies of known duration, and these rank orders were listed under the first, second, third, fourth, fifth, and sixth pregnancies occurring in the Laboratories. Abortions and miscarriages were not taken into account. The average ranks obtained (rank 1 being assigned to the longest gestation) are as follows:

FIRST PREGNANCY (N = 13)	SECOND PREGNANCY (N = 13)	THIRD PREGNANCY (N = 10)	FOURTH PREGNANCY (N = 6)	FIFTH PREGNANCY (N = 2)	SIXTH PREGNANCY (N = 2)
1.9	2.3	2.8	2.9	2.0	5.5

In interpreting these data the following circumstances must be given consideration: (1) Whereas some individual records conform closely to this trend, others are quite inconsistent. Thus Soda's successive terms of pregnancy, starting with the first, are 235, 232, 230, and 228 days; those of Nana are 217, 230, 226, 234, 234, and 216 days. The tabulation indicates an average tendency. (2) It is possible that there have been some influential long-time changes in living conditions (e.g., improvements in nutrition, increasingly crowded caging facilities). (3) During the past 4 years infants have been removed from their mothers soon after birth, thus eliminating the

nursing period and appreciably shortening the interval between successive pregnancies, whereas previously the chimpanzee mothers usually were allowed to keep their infants for at least a year.

It may be noted that this indication of a relationship between gestational length and the ordinal of pregnancy or age of the chimpanzee mother is contrary to the findings of Hotelling and Hotelling ('32, p. 656) in analyzing human data: "No significant difference appears either in mean length or in standard deviation between first and later pregnancies. Age of the mother seems likewise to be without effect."

GENITAL SWELLING AND BLEEDING DURING PREGNANCY

Among the last twenty-nine cases, the number of sexual skin swellings reaching maximum tension during pregnancy ranged from 0 to 4, with an average of 1.31. Most of these swellings occurred during the first 3 months of pregnancy, although a few extended into the fifth and sixth months of the gestational period. Most of the females showed, in addition, minor swellings (not reaching maximum tension). In only one case (no. 23) of the twenty-nine was there no swelling at all during the period of gestation. The only observed instance of bleeding during pregnancy occurred on the twenty-sixth day after the estimated date of conception in case 4.

BEHAVIOR MODIFICATIONS DURING PREGNANCY

"The female during pregnancy quite obviously becomes less active, progressively more cautious and self-protective, better tempered, and more gentle, patient, friendly, and ingratiating with human attendants. Whereas previously she may have been mean, irritable, or pugnacious, with the progress of gestation she ordinarily becomes coöperative. Behavioral indications of malaise sometimes appear in the early months of pregnancy, and appetite may be unstable. . . . It is our impression that the change in disposition tends to be more marked, or at any rate more readily observed, in the primiparous than in the multiparous individual." (Yerkes and Elder, '37, pp. 42 ff.)

These statements are well supported by observation of the last twenty-nine cases. Whereas indications of malaise are most frequent during early pregnancy, most of the noted behavioral changes are particularly conspicuous during the last month or two of gestation and persist for variable periods after parturition. Schultz and Snyder ('35, p. 201) also noted that the behavior of their chimpanzee, Evo, became "a good deal more gentle" towards the end of pregnancy. In general the chimpanzee mother returns more quickly, sometimes within a week, sometimes after a month or two, to her usual (prepregnant) type of behavior when the infant is removed shortly after birth than when she is allowed to keep and to nurse the youngster. In the latter case much of the behavior characteristic of late pregnancy may persist during the entire nursing period (a year or longer).

The attitude of the parturient chimpanzee to human contacts is in many respects very similar to that of the sick or injured animal. In both cases the behavior might be interpreted as deriving from a greatly enhanced feeling of dependence, the individual being no longer so self-sufficient and having greater need for help, sympathy, and protection from its social environment.

INDICATIONS OF APPROACHING PARTURITION

Abdominal contour as indication of developing pregnancy varies greatly among individuals, and is not correlated with weight, either of the mother or of the infant at birth. In some, an unmistakable bulge appeared as early as the fifth or sixth month, whereas others did not "look" pregnant on the very day of parturition. In many instances the vulva increased in apparent size about 2 weeks before parturition. Increased frequency of urination was often noted hours, days, or weeks before parturition.

DIURNAL AND SEASONAL DISTRIBUTION OF BIRTHS

In twenty-three of the forty-nine cases birth occurred between 6.00 A. M. and 6.00 P. M.; in the other twenty-six cases it occurred between 6.00 P. M. and 6.00 A. M.

There has been at least one birth in each month of the year:

JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.
7	3	4	8	7	5	4	1	2	4	3	1

No significance may be attached to the particular seasonal distribution indicated here, inasmuch as matings were controlled and arranged in accordance with the experimenters' convenience and objectives. Although Young and Yerkes ('43) have found that season has an effect on the length and other characteristics of the sexual cycle, the above data do show that oestrus and fertility in the chimpanzee are not sharply restricted to certain months of the year.

PARTURITIONAL PROCESS AND PLACENTOPHAGIA

Parturition was observed in nine of the twenty-nine new cases. Vertex presentation was determined with reasonable certainty in seven of these. In one instance the occiput was turned slightly from the anterior; the anterior-posterior position of the head was not determined with certainty in the other six cases. Combining these with the earlier observations (Elder and Yerkes, '36, p. 418; Yerkes and Elder, '37, p. 45), there are eleven cases in which vertex presentation was determined, of which two were with occiput anterior and two with occiput posterior.

The duration of labor ranged from 40 minutes to 8 hours. In general primipara had more prolonged labor than multipara. Some pain and distress was indicated in all nine cases; the symptoms were intense in cases 6, 8, and 14. In some instances labor began with a small amount of bleeding, and in all cases there was some loss of blood and other fluids (varying from much to little) during the process. Grunting while in a squatting position, often preceded by a quick, short run, frequently was the only indication of contractions, although these sometimes could be seen as sharp movements of the abdomen. In general the contractions increased in rate as labor progressed, but there was considerable irregularity and in two cases they seemed to decrease in intensity and frequency just

before birth. First appearance of the head in the birth canal was followed by completed birth within 4 minutes in all cases except no. 8 where the interval was 22 minutes.

Ordinarily the placenta is expelled within an hour or two, sometimes within a few minutes, after birth. In a few cases it was delayed as long as 12 hours.

All or most of the placenta was eaten in thirteen of the twenty-nine new cases, small bits of it in four cases, and none of it in ten instances. Observations were inadequate in two cases. Combining these findings with those previously reported, it appears that placentophagia occurred in a little over one-half of the forty-nine parturitions. Under more natural conditions this percentage would probably be appreciably higher. At least some of the fluids lost during parturition (on the floor and adhering to the infant) were licked up by all mothers observed. It is certain, however, that eating of the placenta is not essential for the occurrence of lactation. There is a low and unreliable positive correlation between placentophagia and the strength of the chimpanzee mother's attachment for her infant.

INITIAL RESPONSES OF THE CHIMPANZEE MOTHER TO HER INFANT

Previous observations have indicated that whereas the multiparous individual usually shows marked possessiveness and skill in taking care of her infant, the primiparous female sometimes manifests indifference or fear of the newborn and handles it clumsily or not at all (Tinklepaugh, '32, p. 197 ff.; Yerkes and Elder, '37, p. 46 ff.; Yerkes, '40). The twenty-nine cases since July, 1937 in general confirm the earlier findings.

Bimba (case 6) appeared to be terrified at the initial sight of her newborn infant Banka. In avoiding the new, strange object, she made a leap of 5 feet or more, which tore the umbilical cord, and she then fled to the furthest corner of her cage. Although Banka was removed at once and permanently, so that Bimba had a minimum of experience with the infant, her behavior with her second-born was quite different. She

picked up the infant Brinka (case 7) within a few seconds after birth and kept it on her abdomen thereafter. She was very slow in cleaning it and for the first day or two her handling of the baby was somewhat awkward. Nursing was first observed on the second day after parturition. Her care of Brinka during the subsequent 6 months was entirely adequate.

Bula's behavior with Karla (case 8) was in all essentials similar to that of Bimba with Banka. Bula screamed when Karla was born and kept as far away from the infant as possible. As soon as the placenta was expelled, about 5 minutes after the birth, Bula ran away and the infant was removed.

Bentia (case 4) was not observed until 30 or 40 minutes after the birth of Stib, at which time the infant was dead. She paid no attention to the lifeless body which was promptly removed. The birth of Bentia's second baby (case 5) also was not seen, but about 6 hours later she was found carrying the infant rather awkwardly. It was quite dry and clean. After a small dose of sedative ($4\frac{1}{4}$ gr. Nembutal) had been administered, a pistol was suddenly fired at a moment when Bentia had partially relaxed her hold on the infant; she made a dash for the adjoining cage, leaving the infant behind. Evidently her motivation to keep in contact with the infant and protect it was not very strong.

Alpha's rather inadequate behavior towards her first-born has been described (Yerkes, '40). Although Alf was removed within 7 hours, and her experience was thus quite limited, Alpha showed considerably greater skill in handling her next two infants, and separation of mother and infant presented much more difficulty.

All other births occurring since July, 1937 involved multiparous females and with one exception our observations indicated adequate care of, and a strong drive to retain possession of, the infant. In most of these cases, however, the infant was removed from its mother shortly after birth, thus greatly limiting the extent of our observations. Cuba's infant Cub, her third-born, was not seen until approximately 12 hours after parturition. At that time he was being carried, rather care-

lessly, by Cuba's cagemate Lita, a young adult female who has had no infants of her own. Cuba seemed entirely indifferent to both Cub and Lita. No trace of placenta or umbilical cord was found and it is not known whether Cuba or Lita consumed them. After the removal of Cuba, Lita was induced, without the use of anaesthetic, to give up the infant.

Observations of the mother's behavior towards the infant in forty-nine cases may be summarized as follows: (a) In general the multiparous female manifests a more immediate strong attachment to her infant, and especially greater initial skill in caring for it, than does the primiparous individual. Avoidance or completely inadequate care of the infant occurred, as far as is known,² only in the case of a female's firstling. (b) Certain individuals (e.g., Nana, Soda, Fifi, Dita) have regularly given their infants, including their first-born, better care than certain other animals (e.g., Wendy, Cuba).

How much the chimpanzee mother's behavior may be affected favorably or adversely by the presence of human observers and other conditions of captivity, and to what extent it may be influenced by the interest, help, and example of other, experienced females present during and after parturition, are at present matters of conjecture.

POST-PARTURITIONAL BLEEDING AND RESUMPTION OF SEXUAL CYCLES

Bleeding from the vagina between 10 and 28 days after parturition occurred in seventeen of the twenty-nine cases; the duration of such bleeding varied from 1 day (a single observation) to 8 days. In addition, there were five cases in which it occurred only during the first 9 days postpartum. The average duration of bleeding in the twenty-two cases observed was 3.7 days. Since the animals ordinarily were examined only once a day, brief or scanty bleeding may have been overlooked. Elder ('39) who observed post-parturitional bleeding in fifteen cases in our laboratories has reported on a

² Whether Cuba abandoned Cub (case 11) or whether Lita abducted the infant is uncertain.

total of eighteen recorded cases. Evidently the phenomenon is common, but its interpretation remains uncertain.

Among the eighteen (out of twenty-nine) instances in which the infant was separated from its mother shortly after birth, the first menstruation followed parturition by about 3 months (range $1\frac{1}{2}$ to 5 months). The interval was not appreciably longer when the mother chimpanzee nursed its infant 6 weeks or less (four cases). One mother (case 32) nursed her infant 12 months and first menstruated 6 months after parturition. In the other instances of nursing for 6 months or longer, the first menstruation occurred from $2\frac{1}{2}$ to $4\frac{3}{4}$ months after separation from the infant.

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FURTHER STUDIES CONCERNING THE ACTION OF SODIUM CHLORIDE ON THE PITUITARY

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ONE PLATE (SIX FIGURES)

In a previous investigation it was shown that following intravenous administration of hypertonic sodium chloride solutions, distinct morphological changes occur in the pituitary of the rat. These are characterized by the accumulation of large amounts of fluid in the pituitary cleft between the middle and the anterior lobe and by a vacuolization of the anterior lobe basophils which eventually tend to degenerate and to form cystic cavities (Selye, '43). In view of the important role played by the pituitary in connection with water and salt metabolism, it was decided to extend these observations and to determine whether an increase in the dietary salt intake would also elicit detectable changes in hypophyseal structure.

Eighteen male albino rats weighing 60-105 gm. (average 89 gm.) at the beginning of the experiment, were subdivided into two groups. All of them were fed purina dog chow ad lib. but six of them were allowed to drink tap water while the remaining twelve were given only NaCl solution. At the onset of the experiment the NaCl solution was 1.5%, after 2 weeks this was raised to 2% and after 4 more weeks to 2.5%. Rats given very high concentrations of NaCl instead of drinking water usually refuse such solutions, but in our experiment the animals took large amounts, probably because they were allowed to adapt themselves gradually to the salt water. The fluid consumption and output showed great individual varia-

¹ Work performed during the tenure of a National Research Council Studentship.

tions, but while the controls excreted about 1-4 cc. per day on the average, the salt treated rats eliminated 35-50 cc. daily. Four animals in the salt treated group died during the course of the second month of the experiment and all the remaining rats were sacrificed at the end of the second month.

At autopsy the pituitaries of the salt treated rats were distinctly different from those of the controls. The posterior lobe appeared to be edematous and protruded markedly above the surface of the organ. The middle lobe — which is clearly detectable by naked eye inspection as a light ring separating the anterior from the posterior lobe — appeared to be extremely thin and inconspicuous. The anterior lobe, on the other hand, was perhaps even somewhat smaller than normal (figs. 1 and 2). These changes in appearance did not lead to any significant difference in the total weight of the organ which averaged 5.6 mg. in the salt treated and 5.4 mg. in the control animals. It must be remembered, however, that at the end of the experiment the untreated controls were almost twice as large as the salt treated rats whose development was of course inhibited by the excessive NaCl treatment. The average body weight of the controls was 190 gm. (range 180-205 gm.), while that of the NaCl treated animals was 107 gm. (range 95-160 gm.). In view of this difference in body weight the relative weight of the hypophysis was greater in the salt treated than in the control animals.

Upon histological examination it became evident that the most pronounced hypophyseal changes induced by salt treatment occur in the posterior lobe tissue. Even under low magnification this lobe appeared to be diffusely imbued with lightly staining edema fluid and in spots it exhibited a honey-combed appearance due to the presence of numerous vacuoles in the otherwise firmly packed posterior lobe tissue. Upon closer study, especially under high magnification, these small vacuoles were found to result from the presence of numerous pituicytes which were greatly enlarged and in the process of active mitotic proliferation. During mitosis the texture of the cell becomes extremely loose so that the cytoplasm tends to

shrink away from its surroundings and often cells are washed in the course of the staining. In a good many cases, however, the cells engaged in mitosis remained attached to the slide and could readily be identified. The outlines of such cells were exactly the same as those of the empty vacuoles. The presence of many partly shrunken cells whose body was detached from the remaining tissue on one or two points only, confirmed our view that the honey-combing of the posterior lobe is an artifact resulting from the occurrence of numerous cells in mitosis which are lost in the preparation.

The middle lobe appears to be very thin in all the NaCl treated rats and consists only of a few layers of cells. In some cases the cleft between the middle and anterior lobes was greatly distended by fluid just as it was in the previously mentioned acute experiments in which the hypertonic saline solution was administered by the intravenous route. In other cases, however, the cleft was of normal appearance. The borderline between middle and posterior lobe showed certain irregularities in structure which appeared to arise from the degeneration of middle lobe cells in this area.

The anterior lobe revealed no constant pathological change, but in the great majority of the NaCl treated rats the basophils were extremely prominent both in size and in number.

The occurrence of these pituitary changes is of special interest, firstly because mitotic divisions are rarely found in posterior lobe tissue under normal conditions and secondly, because they show that exogenously induced changes in salt and water metabolism may secondarily influence the hypophysis perhaps because they elicit a compensatory defense reaction.

Perusal of the literature revealed no observations comparable to those reported above, but it is perhaps pertinent to mention that according to Hashimoto ('37) diuretics, such as caffeine or diuretin, increase the basophil-count of the anterior lobe of the guinea pig. Similar observations have been made by Goldzieher and Kaldor ('30, '31) and Hashimoto ('37) with novasurol in the same species.

SUMMARY

Experiments performed on albino rats indicate that if concentrated NaCl solutions are given instead of drinking water over a period of several weeks, definite changes appear in the hypophysis. These are characterized by a macroscopically detectable swelling of the organ and the appearance of numerous mitotic figures in the posterior lobe tissue. Simultaneously the middle lobe decreases in size apparently owing to degeneration of many of its cells. Less constantly there appears to be an increase in the fluid content of the hypophyseal cleft and in the number and size of the anterior lobe basophils.

ACKNOWLEDGMENTS

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PLATE 1

EXPLANATION OF FIGURES

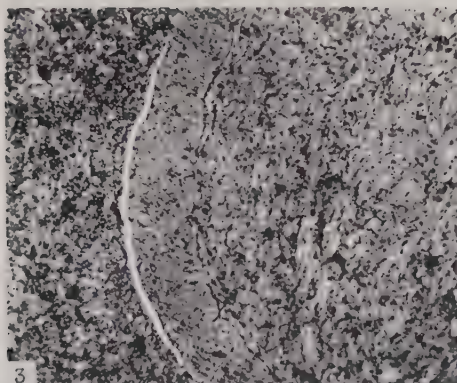
- 1 Macroscopical view of the hypophysis of a control rat.
- 2 Macroscopical view of the hypophysis of a NaCl treated rat (same magnification as fig. 1). Note prominent and enlarged posterior lobe and atrophic, thin intermediate lobe.
- 3 Section through anterior, middle and posterior lobe of a normal control rat.
- 4 and 5 Section through anterior, middle and posterior lobe of NaCl treated rats (same magnification as fig. 3). Note that the intermediate lobe is atrophic in both cases, but in the pituitary shown in figure 4 the cleft is greatly distended and the posterior lobe is imbued with a light edema fluid while in figure 5 the cleft is normal and the posterior lobe appears "honey-combed" because of the presence of numerous vacuoles and light cells in mitosis.
- 6 High magnification of a cell in mitosis taken from the posterior lobe of the rat shown in figure 5.



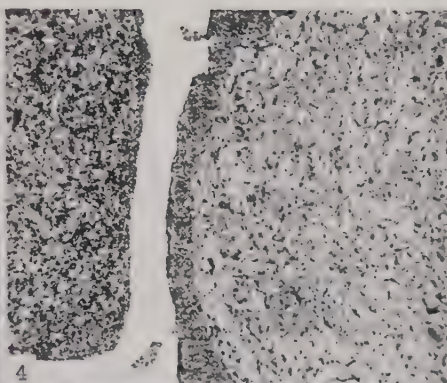
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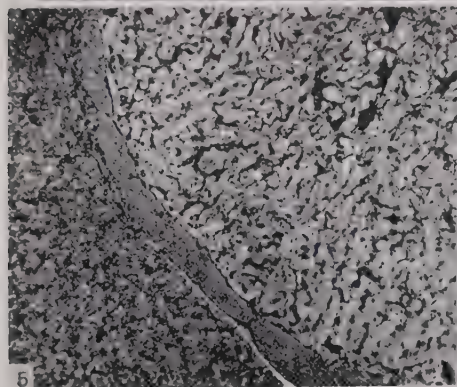
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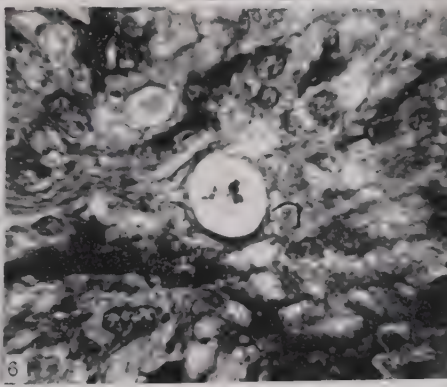
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BOOK REVIEW

BIOCHEMISTRY AND MORPHOGENESIS. Joseph Needham. At the University Press, Cambridge. Macmillan Co., N. Y. xvi + 785; 1942.

This book, though not a continuation of Needham's *Chemical Embryology*, published in 1931, is still concerned with the great problem of the relation between morphology and biochemistry. Needham in his general introduction recognizes three possible approaches to this problem: (a) by making an attack on the organization level lying between the largest chemical particles and the smallest known morphological structures, (b) by an examination of the chemical changes which go on during change in form of the developing embryo and (c) by a biochemical investigation of the so-called morphogenetic hormones. The present work is divided into three main parts: Part 1—The morphogenetic substratum; Part 2—The morphogenetic stimuli and Part 3—The morphogenetic mechanisms. The remaining pages of the book comprise a brief general conclusion, a glossary of some of the special terms used, a bibliography, a general index as well as indexes to the scientific names of animals and plants and the kinds of genes referred to in the text. (Indexes compiled by Margaret Miall). It is illustrated by 36 plates (including frontispiece) 4 of which are in color, and contains 328 figures and 32 tables.

The first part, the shortest of the book, is an account of the main advances made since 1931 in the knowledge of the chemical raw materials from which the embryo is built. Attention is first directed to the problem of "the way and order" in which each chemical constituent is laid down in the maturation of the egg in the ovary as a basis for comprehending the nature of the properties of polarity and symmetry. Much of the work reviewed pertains to the histo-chemical nature of the egg of birds, especially of the hen (including the yolk, the white, shell and shell membranes and the vitelline membrane and its physiology). The newer work on the effects of such factors as storage temperature, length of storage, properties (amount) of egg white, porosity of the egg-shell, protein diet, and vitamin content on the hen's egg before development has started as manifested in hatchability. It is disappointing to note, as the author points out, that no studies with modern techniques have been made on the

chemical constitution of the amphibian embryo, which has been so very important in elucidating the nature of morphogenesis in embryonic development.

The author next brings together in an original way the literature on the degree to which eggs of different organisms are dependent upon external environment — the extent to which the egg is supplied with materials sufficient for complete development and to what extent the egg is dependent upon extrinsic materials essential for its development into an embryo. The work of Ranzi and his collaborators, especially on selachian fishes, has been fully given to illustrate “all the stages between dependence on the environment as regards water and salts, through approximate equilibrium, to dependence on the maternal organism as regards water, salts and organic substances” (p. 38). Finally he examines the mortality curves of avian development in order to throw light on the nature of embryonic metabolism of the cleidoic egg; the rôle of the temporary structures, the yolk sac, amnion and allantois, in the course of embryonic nutrition in birds; and the biochemistry of the mammalian placental mechanism. With these the author completes the story of the nutritional substratum of morphogenesis.

Part II, the longest in the book, is broadly speaking an analysis of morphogenetic stimuli, those factors which operate from one part of the embryo to another during development in the production of specific form. It begins with general concepts of causal embryology including very properly a history of the idea that chemical substances are involved in correlative development.

Next an attempt is made to appraise biochemically mosaic eggs, those in which regional differences in the cytoplasm are more or less determined at the time of fertilization. This is followed by a long section on the fundamental principles of morphogenesis derived from an experimental study of amphibian development. Here are included an account of the nature and mechanism of the formative movements of gastrulation; the biological and chemical properties of the primary organizer (dorsal lip) and its rôle in the establishment of the vertebrate axis; regional differences in metabolism of the gastrula and their bearing on the liberation of the primary evocator from its inactive precursor and the response of the reacting tissue; anomalies resulting from inhibition of organizer function; the necessity for a doubling of the organizer in twinning; the formation of teratomata as a result of un-coordinated organizer action (failure of “individuation field” to control the action of evocating substances — p. 233); speculation on ways in which embryonic organizers are possibly connected with the problem of cancer (p. 243); and regional differentiation, including regional specificity of the organization center and of other

organ-forming parts of the embryo, second grade organizers (i.e., induction of lens by the eye cup, limb bud induction, gonad induction, etc.).

The next two sections are short, dealing respectively with organizer phenomena in prochordates and in vertebrates other than amphibians, and with temperature effects on determination. Needham next discusses the relation of genes and organizers, in which are treated such outstanding topics as non-species-specificity of organizer action, interference of lethal and sub-lethal genes on organizer action and the resulting morphological effects, and genic control of chemical and morphological processes. This section of the book is completed by an examination of regeneration and the rôle of inductors of the endocrine type in amphibian metamorphosis, and a study of organizer effects in insects and echinoderm development. Regeneration of the limb or tail in the Amphibia involves dedifferentiation in the stump and a repetition of ontogenetic processes similar to those proceeding in normal development of the organ. What little is known of the biochemical aspects of regeneration is presented in a way so as to challenge further investigation.

The treatment in this part of the book will be of especial interest to many readers and investigators, since it falls directly in the field where the author and his co-workers have made significant progress toward an elucidation of the biochemical nature of the organizer and its mode of action.

The third part of the book has as its objective the physiological mechanisms operating in morphogenesis. First under the heading "Dissociability" is considered the lack of dependence on the integrity of the whole of the processes of growth, differentiation and metabolism, i.e., the occurrence of growth without differentiation and vice versa, of metabolism without growth and differentiation, etc. These phenomena may have value in connection with the problem of malignant tumors and developmental arrests or malformations which result from the failure of some morphogenetic process.

The reader is next given an account of the problem of heterauxesis, which is defined as "the relation of the growth rate of a part of the developing organism (whether morphological or chemical) to the growth rate of the whole, or of another part." An analysis of growth ratios of chemical constituents suggests that animals of very different morphological form and size have "the same basic general chemical plan of growth, which is deformable within wide limits in space-time." The literature on respiration and metabolism of the developing embryo is reviewed so as to furnish a basis for a future understanding of how the chemical transformations and energy changes proceeding during development are integrated with the morphological changes

and the differentiation process. This part of the book is concluded with speculations on polarity and symmetry, two enigmatic properties of the egg and embryo as a basis for understanding how morphogenesis conforms to them. These are treated from the standpoint of the ultra-structure of cell protoplasm and its connection with morphogenetic processes.

The above description gives a résumé of the content of the volume. Needham has brought together here an extensive literature and has shown a high degree of success in relating the chemical and morphological data to one another and in showing the kind of problems that are involved in the change in form of a developing embryo. The exposition is excellent and the argumentation convincing yet some question must be raised concerning the extent to which he has critically evaluated all of the available data. It is a prodigious task to read and critically evaluate all of the literature dealt with. My point here is illustrated by the short section on Sex Determination (pp. 310-319) which falls within the scope of my own special interests. In the account of sex reversal in chick embryos induced by sex hormones he states "But when testosterone propionate was similarly introduced by Danchakova, the embryos could not survive it." This misleads the reader for when relatively large doses (2.4 mg.) of testosterone propionate are administered, the embryos not only survive but, if female, each gonad is modified into an ovotestis, the oviducts are almost completely degenerated and the Wolffian ducts are greatly hypertrophied (The paper containing these results is cited in Needham's bibliography!). Also "Conversely, Danchakova was able to produce full masculinisation of the mammalian embryo (guinea-pig) by introducing testosterone propionate in an oil-drop into the amniotic cavity very early in gestation." Most readers will interpret "full masculinisation" to mean that a genetic female had been completely modified into an anatomical male. This is not the case, since according to Danchakova, in genetic females so treated the potentially male sex ducts and accessory glands and phallus are made to persist and develop, and the gonad primordium develops as a "strong stimulated ovary" and both Fallopian tubes and uterus persist although not entirely unaffected by the male hormone. In still another line "For the mammalian embryo, oestrone, Danchakova claims in doses sufficient to give sex reversal, is quite lethal," Needham shows again not to have all of the facts at hand. His account does not include any synthesis of the researches of Green and his collaborators on the rat, Raynaud on the mouse and Burns and Moore on the opossum, in which organisms these investigators have shown that the embryonic gonad is not significantly affected by either estrogens or androgens although the doses administered are sufficient to produce marked

modifications of other sex structures. No reference whatsoever has been made to the investigations of Burns and of Moore on sex reversal in the opossum and incompletely to those of Greene et al. and of Raynaud. Likewise, in the case of reptiles, as the reader will note in table 23, p. 314, no reference is made to the studies of Forbes on the alligator. The account of sex reversal as induced in the amphibian embryo is decidedly a one-sided view, little or no incorporation of the careful analyses of Burns and Humphrey having been made.

It is not a little surprising to find the important investigations of Lillie and his co-workers on the morphogenesis of the feather papilla, which is so clearly related to the author's central theme almost entirely ignored. The feather papilla has proved to be an embryonic organ par excellence for the analysis of problems in morphogenesis and differentiation. Its course of structural development has been altered by hormones, i.e., the shape of a feather formed by a given papilla may be completely reversed from male to female by the action of female hormones, et cetera, and it has furnished outstanding evidence on the nature of the physiological mechanisms (growth rate, gradients in threshold and reaction time) involved in the control of the differentiation of pigmentation patterns in feathers. Another conspicuous omission, particularly notable in the section on polarity (p. 656), is any reference to the views of Child as expressed in his latest book "Patterns and Problems of Development" ('41). It hardly seems possible that any student of the development of form could entirely ignore this book even though it may arouse controversy in the minds of some.

There are other sections and topics which various readers may criticize. Some may object to his flair for and emphasis on terminology or to his use of the antiquated Milnes-Marshall figures of amphibian gastrulation (fig. 55) when more accurate figures of Vogt and Spemann are available; others to some of his definitions of terms as expressed in the glossary (i.e., when he says "the bird's egg has no chorion"); and still others to the encyclopedic or review character of much of the treatment as opposed to a thorough synthesis and critical evaluation of the literature.

Nevertheless, with all of the criticism that may be directed against it, Needham's book remains an extremely stimulating and useful one. It is a provocative book and for years to come the ideas expressed in it will serve to stimulate new ideas and investigations on the production of form through chemical processes, a field in which there has been a recent revival of interest owing to the advances made in our knowledge of the molecular structure of proteins. It will be of special value to anatomists, since among them there has been far too little appreciation of or inquiry into the problem of organic form. It will

serve to stimulate interest in the problems of the genesis of the form of organs and other parts of the body, and of the chemical basis of anatomical form. There is scarcely a textbook on developmental anatomy that even calls attention to the problem of the causal factors concerned in gastrulation and in the folding of germ layers to form organs like the brain, the spinal cord and the liver. We have been for too long merely content to study anatomical form without much consideration of the physico-chemical nature of the matter from which it is inseparable. The book has fulfilled admirably well its whole object which was, in the words of Needham, "to show that the fields of chemistry and morphology are not so sundered as is often supposed."

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BOOK REVIEW

DIAGNOSIS OF UTERINE CANCER BY THE VAGINAL SMEAR. By GEORGE N. PAPANICOLAOU AND HERBERT F. TRAUT. The Commonwealth Fund, New York. vii + 46 pp. 1943 (\$5.00).

About a quarter century of first-hand observations upon vaginal smears has afforded a desirable and fundamental background for attempting to use such smears in diagnosing uterine cancer. The results of intensive investigation for three years are recorded in this monograph. Observations were made on 7,000 to 10,000 smears from the vaginae of 3,014 adult women most of whom were at the cancerous age of life. In this group, 193 instances of carcinoma of the uterus or some portion of the lower reproductive tract were encountered. Final diagnosis was made histologically. In all cases except eleven, malignant cells were identified in the smears. Previous estrogenic therapy does not hinder diagnosis; instead, it facilitates the recognition of cancerous cells by inducing a cornification of normal cells. In nine women, smears were responsible for the detection of previously unsuspected cancer; sometimes multiple curetting over a period of months was required before a positive histological diagnosis could be made. Yet, in spite of their success, the authors judiciously state that the vaginal smear technique ". . . is not recommended as a means of ultimate diagnosis. It should be used as a preliminary or sorting procedure and should be confirmed as a matter of routine by biopsy and tissue diagnosis."

Malignant cells originating in tumors of the bladder, the urethra and the vulva were observed in vaginal smears. This is rather surprising because the vaginal samples were collected from the posterior fornix. Referring to other mucous passages, the authors make the interesting suggestion that ". . . it would seem quite possible that in time the cancer cell may be routinely recognized in fecal, urinary, and gastric secretions"

Anatomists and endocrinologists will be interested especially in the morphological variations to be encountered in smears from non-cancerous individuals. Eleven pages are devoted to the following: a description of the normal cellular constituents of human vaginal fluid; the distribution of the cellular types at various stages of the sexual cycle. It was noted that, except under pathological conditions,

endometrial cells rarely are found in the vagina after the sixth day of the menstrual cycle. The "Effect of Modified Physiological and Pathological Conditions on the Cellular Contents of the Vaginal Fluid" is discussed in Chapter 4, seven pages being devoted to Post-partem Period, Abortions, Ectopic Pregnancies, Prepubertal Stage (including the newborn), Menopause, Amenorrhea, Hyperestrin and Endometrial Hyperplasia, Infections and Some Other Conditions. Previously unpublished data are included in the second, third and last of these subdivisions.

All illustrations are in color, the colors being those yielded by a single method of staining which in thick or bloody smears provides greater cytoplasmic transparency and permits more intense staining of the nuclei. Four and one-half pages deal with the preparation and staining of smears. The eighteen colored photomicrographs, favorably magnified and arranged on three plates, are excellent as are the remaining eight plates which contain a total of 281 colored drawings, all having the same magnification. Unfortunately their arrangement does not permit rapid comparison of normal and abnormal cellular types. Thus in order to compare the various cells of a menopausal subject having cervicitis with those of a patient having squamous cell carcinoma of the cervix, the reader is obliged to consult five plates.

From the point of view of the fundamental sciences, the authors are to be commended highly for originating the cellular approach to this problem, for observing meticulously and interpreting objectively an enormous amount of material, and for presenting the whole in concise form. From a practical standpoint, this book will provide clinicians with substantial supplementary information, although the early recognition of cancer will continue to require competent diagnosticians with extensive experience. Probably, however, judgement should be postponed as to whether this "sorting procedure" is destined to become a routine diagnostic method. Finally, an outstanding contribution of the book is the encouragement which it offers to revolutionary approaches in fields of investigation other than uterine cancer.

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PROCEEDINGS
OF THE
AMERICAN ASSOCIATION OF ANATOMISTS

1943

The following seven memoirs of deceased members of the American Association of Anatomists were prepared by committees appointed by Acting President J. P. Schaeffer.



Edgar Allen

EDGAR ALLEN

1892-1943

The sudden death of Edgar Allen on February 3, 1943 took from us the President of our Association and from the world a distinguished anatomist.

Dr. Allen was born at Canyon City, Colorado, May 2, 1892. He was the son of a physician. The family soon removed to Providence, Rhode Island. Here Edgar Allen received all his formal education, and here, on the waters of Long Island Sound, he learned the love of sailing and of the sea which gave him many of his happiest days and helped, no doubt, to develop his characteristic traits of courage, frankness and resourcefulness.

He began early to specialize in biological studies, taking the Ph.B. degree at Brown University in 1915, his M.A. in 1916, and the Ph.D. in 1921. This period of university training was interrupted by the first World War, during which Allen served in France, first as a volunteer in the Brown University Ambulance Unit and later in the Sanitary Corps. At the time of his discharge, in 1919, he held the rank of 2nd lieutenant. Before leaving for France in 1918 he married a fellow student at Brown, Marion Pfeiffer, who was his devoted lifelong companion. There are two daughters of this marriage.

After the war he became a junior member of the Department of Anatomy of Washington University, St. Louis, where he began the studies on the physiology of reproduction and the histology of the reproductive organs which were destined to bring him a great reputation and to open a new chapter of research. In 1917 Stockard and Papanicolaou had introduced

their method of following the reproductive cycle of the guinea pig by means of the vaginal smear, and had greatly clarified our knowledge of the cycle of that species. Long and Evans had promptly applied the new technique to the rat with brilliant results, and Allen applied it to the mouse. In 1921 his thesis "The Estrous Cycle in the Mouse" was accepted by Brown University for his doctor's degree. In the course of this work he was led inevitably to ponder upon the remarkable coördination of events in the ovary and uterus, and to conjecture that the ovarian follicles may contain a hormone that causes the estrous changes in the uterus and vagina. This hypothesis was tested by the simple method of injecting filtered follicle fluid from swine into castrated mice. The prompt and striking induction of estrous changes in the mouse vagina showed Allen that a hormone was indeed present in the follicle, and provided a practical means of assaying it. In the investigations that followed, Allen provided the biological techniques and E. A. Doisy contributed the skillful chemical analysis which soon made possible the isolation and chemical identification of the estrogenic hormones.

His subsequent studies all dealt with problems of the ovary, the ovarian hormones and their relation to the reproductive cycle, to the normal growth of tissues, and to abnormal growth such as that of cancer. His demonstration that removal of the ovaries of the rhesus monkey generally causes menstruation-like bleeding gave an important clue to the problem of the menstrual cycle. With the coöperation of Dr. Jean Paul Pratt of the Henry Ford Hospital, Detroit, Q. U. Newell and L. J. Bland, he secured in 1930 the first human ova from the oviduct, and thus provided valuable evidence with regard to the time of ovulation in man. After 1933 he became more and more interested in the problems of cancer, especially in relation to the sex-gland hormones. The list of his contributions is far too long to be cited here; a glance at the index of any current book on the physiology of reproduction will show the breadth of his interests and the extent to which he influenced the work of his colleagues throughout the world.

Allen's success as an investigator, his personal charm and vivid character brought him rapid promotion. Called to the University of Missouri in 1922 as professor of anatomy he became associate dean of the medical school in 1929 and dean the following year. In 1933 he was appointed professor of anatomy at Yale University School of Medicine. Here he quickly organized a large and active group of young investigators of whose achievements he was very proud.

Allen was a striking figure personally, for his broad shoulders, ruddy face and snow-white hair made him conspicuous in any group. He made friends quickly and was always the first to encourage and to admire good work by others. His boyish frankness and his good will contributed more perhaps than any other factor to the effective cordiality which prevails among the American workers in the physiology of reproduction, for he set an example of scientific truth-seeking untarnished by pettiness and jealousy.

His enthusiasm for sailing was characteristic of the direct, energetic manner in which he threw himself into anything that interested him. He enjoyed administrative tasks and the associations they made for him. He rarely chose to work alone on a scientific problem, for his excitement and enthusiasm reached their highest peak in team work. Most of his investigations were done in collaboration with younger colleagues to whom he gave full support and generous credit.

These qualities served him well in the organization of a brilliant group of colleagues all over the country who contributed, under his editorship, to the admirable handbook "Sex and Internal Secretions."

He did not lack recognition of his services. Three of the universities with which he was associated, Brown, Washington University, and Yale, awarded him honorary degrees. He was enrolled in the Legion of Honor on the occasion of a scientific meeting in Paris in 1937 and received the Baly Medal of the Royal College of Physicians of London in 1941. In the last year of his life he was president of the two national societies most closely representative of his fields of work, the

Association for the Study of Internal Secretions and the American Association of Anatomists — honors conferred by those who best understood his work. His untimely death prevented him from actually directing a meeting of the Anatomists as president; but many members will recall his genial and effective service as acting president at the meeting of 1931, held at Northwestern University, when he was vice-president, the president being absent.

Allen's friends were aware that he had suffered a severe coronary attack about 10 years ago; but he faced this warning with outward calm and went about his work. Nothing could have kept him from research nor from his sailboat. When war came upon us again he insisted upon joining the Coast Guard for a regular weekly tour of duty on Long Island Sound, as operations officer of a flotilla. It was during one of these patrols that he died suddenly of coronary occlusion. Such an end to active life, on his beloved blue water and serving his country in time of war, was fitting to his own sense of duty; but he will be remembered best for his ardent service in the war that never ends, of Mankind against the Unknown.

GEORGE W. CORNER
C. H. DANFORTH
L. S. STONE



Con. Swell

FRANCIS HUNTINGTON SWETT

1893-1943



The difficulties of writing a life sketch of one who presented so many facets to his nature are accentuated when the writers are intimately aware of the protagonist's critical attitude with its honest and forceful expression. No matter how simply the sketch be phrased, it is taking advantage of its subject who would lightly toss aside its words of praise as not to be taken seriously by himself or others.

Francis Huntington Swett was born November 13, 1893 in Norway, Maine (which he used to remark was situated between Paris and Berlin). It was here that he absorbed the philosophy and innate keenness of that region. Its laconic form of speech and expression served him well; its sense of the deeper values of responsibility and service was thoroughly ingrained in his nature and character.

His undergraduate training was received at Bates College from which he obtained with Phi Beta Kappa honors, his A.B. degree in 1916. During his schooling here, Professor Pomeroy first fostered and directed his interests in the field of Biology — a field in which his later research was always centered. In 1917 he went to Brown University, where he assisted in Zoology and where his future interests were further moulded. Mead, Walter, and Mitchell set a standard in teaching and interest which he was always to maintain. He was a student who exacted much from his instructors. Any point carelessly glossed over inevitably stimulated a pertinent question whose answer might clarify the point at issue. His decision was set on teaching as his profession during this period, for his notes

and drawings were all made then with the idea of future use. He acquired from Dr. Mead the method of using plastic models which he employed with a facility that was remarkable to some of his later confreres. He left Brown with two possessions that he prized highly, his M.A. degree and the mutual respect and affection of teachers and pupil.

He spent the following year, 1917-1918, at Tufts as pre-medical instructor in the company of another graduate of Bates and Brown, W. E. Sullivan, then teaching in Anatomy. The friendship of these two Maine men meant more to both than either would ever acknowledge. When the War came, Swett entered the Medical Corps and was stationed at the Walter Reed Hospital. He later was assigned to the Army Neuro-surgical Laboratory at Baltimore. When service experiences were related he would drily remark that he fought the war taking spinal cords out of cats. His acceptance of responsibility combined with his other personal qualities evidently impressed his C.O., Captain Lewis H. Weed, to whom he was later to return in a non-military capacity by request, not assignment.

At the close of the service period Swett came to Yale, where he completed his work for the Ph.D. in 1922. The courses which he took were, as at Brown, completed with the firm conviction that the maximum should be attained for use in future teaching. Harrison, Mendel and Ferris contributed not only to his technical grasp of his field but also to his widening perspectives and deepening knowledge.

The attitude of his teachers and associates is briefly expressed in a Minute of the faculty of the Osborn Zoological Laboratory which reads as follows: "As a student at Yale he was clear headed, slow to speak, critically analytical, both of himself and others. Frank and honest, he cherished simple and direct approaches to his problems both personal and scientific. He hated sham and front. These personal attributes were intensified as he grew in both the scientific and educational worlds."

As a student he belonged to a group which assumed a critical analysis of all student reports and lectures given before the faculty and occasionally, faculty members got a fair share of this treatment. After a long, vague and vacuous spring session which others had vigorously criticized in extenso, Swett's sole remark was, "I have heard better, now I must operate." This simple statement expressed volumes and put the emphasis where it belonged. It was at this same period that "The Book" was put in operation. Any of Swett's friends could get a rapid, accurate and rugged personal diagnosis or evaluation of the great and near great simply by asking the individual's place in "The Book." Many arguments were held over the priorities for the frontispiece in "The Book" which, needless to state, was a place of distinction albeit dubious.

He left Yale as he had Brown, with a background of work well done and the friendship of his teachers and fellow students. All his associations were maintained and one of his greatest charms was his ability to eliminate completely the interval between the past and present. His naturalness obliterated the element of time and with one of his terse comments the conversation began as though the last meeting had never terminated.

His second period in Baltimore began in September, 1922, when he joined Dr. Weed's staff as Instructor in Anatomy in the Johns Hopkins University. During his first 2 years he assisted in the course of microscopic anatomy with Drs. Sabin and Cunningham. In 1924-1925 he taught gross anatomy and was Associate in Anatomy. It was during this period, on July 19, 1924, that he was married to Miss Elizabeth Glenn, of Baltimore, who now survives him. This proved a most suitable and happy marriage. Mrs. Swett is graced with the qualities that made her his capable and understanding companion both in scientific and administrative work as well as in their social responsibilities. In September, 1925, he went with Dr. Cunningham from the Hopkins to the Vanderbilt School

of Medicine, where he remained for 5 years as Associate Professor of Anatomy.

For the Vanderbilt period Dr. Karl Mason has provided a review which reveals the development of the man:

"He always amazed me by his ability to acquaint himself with each new class of students in such a short space of time. Names, personal history and characteristics, good qualities and undesirable ones, all seemed to be revealed to his discerning eye long before they became apparent to others. At the same time, no one could have been more tolerant of the shortcomings of students, or more skillful in calling them to the attention of the student by a few crisp and pertinent remarks at the proper psychological moment.

"Most impressive was his ability to maintain the sternest of discipline whenever necessary and, at the same time, maintain the most informal and friendly relations with his students, technical staff and associates in general. All knew exactly where they stood in Frank's opinion, and both respected and loved him for his frankness and fairness.

"In all of his relationships to others, it was his good humor, ready wit packed into laconic statements so characteristic of a true New Englander, his fine sense of values and genuine friendliness which endeared him to all who knew him.

"He carried out all duties effectively and efficiently, with no indication of rush or urgency. His lectures were clear, concise, simply presented at the student's level of comprehension, and interspersed with intermittent sparkles of dry humor. His talks on his research problems before the Medical Society and other groups, to whom the intricacies of limb bud transplantation were a new experience, were equally brilliant, simply presented and interesting."

Dr. Swett was appointed to the Professorship of Anatomy at the new School of Medicine of Duke University in 1930. He began the department with a staff of three members besides himself and as the school grew he constructed a well diversified organization of seven members. He believed that a working group with varied research interests should be of greater value in a teaching institution than one with interests turned upon a rather circumscribed field. Group research therefore was never a topic under consideration for his department.

The student was his prime interest in the organization here. The thesis that a medical school was primarily a place for training young doctors was uppermost in his mind and in that he received the full cooperation of his colleagues. The methods of instruction he fostered are set forth in his article "Who Teaches Anatomy Anyhow?" The teaching load was heavy and probably greater per man hour than in any other department. However, with all he was eminently thoughtful of the welfare of his department, particularly of the younger members, for whom he had great concern that they get time to pursue their own problems. Post-graduate training was, however, a subject not overlooked, for in future plans for the development of the department, there were places projected for research fellows. The congenial student relationships with the department were, at Duke as formerly at Vanderbilt, in no small way fostered by his wife, who was a special secretary endowed with a capacity of being student confidante and much was brought to his attention which, as student problems, might not have reached him otherwise — at all times despite his abundant duties, his time was always available to the students. He was perfectly willing to discuss anything from minor technical problems to major personal decisions. No matter what their questions might be, his students always knew that they would get from him answers tempered with more insight into and understanding of their problems than from anyone else.

In the academic circles he was often sought for council concerning the premedical problems of education. In the medical school and in the executive faculty he was conceded to have a steady, well-balanced perspective, which damped many a hasty judgment. During the past few years the matter of student admission was handled entirely through his office as chairman of the committee and lately when the Dean was called to Washington, he virtually became the assistant dean, taking over many of his administrative duties. The full influence which he wielded in his quiet, persistent way over students and others cannot be accurately estimated. It forms

an intangible value both to him and to us. The high regard which he engendered was recognized by the American Association of Anatomists in his election to its Secretary-Treasurership.

Swett's interests were particularly devoted to teaching. It was one of his cardinal tenets, however, that continued research is a necessity for keeping alive the enthusiasm of the teacher. To him personally, this was of primary significance with publication as a secondary although obligatory consideration. His comments upon the output of individuals whose slides are still wet at the time of publication indicated that the results tended to duplicate the condition of the slides.

To his own program of research his plans were originally formulated. It centered around the problem of asymmetry and the factors involved in the attainment of asymmetric relations. His first papers dealt with the study of asymmetry in double trout embryos. He early realized, however, that an attack upon the general problem would have to come through the application of experimental method to a part of the organism. Then followed the work on the contribution of each of the quadrants of the limb disc, which formed the background of subsequent studies.

Since the limb provided a logical approach to the problem which he decided to solve, he tested his material from every possible angle. When after one of his presentations on the frequency of reduplication after certain types of procedure, Dr. Stockard asked him what was the cause of reduplication, his answer was, "I don't know; what I did to produce them is just a start on the finding of one of the many factors involved." His refusal to theorize, realizing that only a small part of the evidence was available, was characteristic. The studies on reduplication never satisfied him — the experiments did not provide the critical values which he required. Nevertheless they formed an important part of his scientific background, for they convinced him that certain factors could not be resolved individually by analysis of the free extremity.

In 1928 he began his 12-year study of the girdle and its relationship to the devolving limb. These studies required an intensive and exhaustive amount of work, a tremendous amount of operating and study combined with the most careful analysis of results which at first glance appeared to be heterogeneous. From these, by infinite care and remarkable interpretation, Swett wove the story of limb-girdle interrelation. It is indeed fortunate that he could clarify for himself and for others this information which is prerequisite to future limb studies. The work is not finished and the final answer was denied him. What he did, however, need never be repeated. It is the foundation for the future as he planned it.

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D. C. HETHERINGTON

G. L. STREETER

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COURTESY OF ARCHIVES OF NEUROLOGY AND PSYCHIATRY, 1943

W. G. Ranson

STEPHEN WALTER RANSON

1880-1942¹

Stephen Walter Ranson was born August 28, 1880 at Dodge Center, which is located in the Rochester region of Minnesota, long notable for producing so many outstanding medical scientists and clinicians. He was the youngest of six children. His parents, of English lineage, were deeply interested in education, and gave their children many cultural opportunities, including foreign travel and study. His father, Dr. Stephen William Ranson, had been a teacher for some years before entering on a medical career. The professional training of the father was strong in its influence on this family: three became physicians and one a Doctor of Philosophy in psychology.

After graduation from the secondary schools of Dodge Center, Stephen Walter Ranson entered the state university with the intent of becoming a psychologist. With characteristic acumen he saw the need of thorough grounding in the structure of the nervous system, and this desire brought him early under the influence of Prof. J. B. Johnston. It was a fortunate relationship. The young student admired Professor Johnston greatly and soon found his primary enthusiasm for psychology undergoing a substitution. Through the inspiration afforded by this teacher and investigator independent experiments were initiated by young Ranson in the summers following his sophomore and junior years; a usable laboratory was improvised in the family barn at Dodge Center. It

¹ Presented at the Sectional Meeting of the American Association of Anatomists at Chicago, April 23, 1943.

is interesting that the experimental subjects were cats, since it is well known that this became the animal of his choice in most of his subsequent work. The nature of the experiments is unknown, but of much greater import was the fact that they brought the groping tyro in contact with a book, "The Growth of the Brain — A Study of the Nervous System in Relation to Education," written by H. H. Donaldson. With a glimpse of new, beckoning horizons, transference of residence was made to the University of Chicago and in 1902 credits were completed by which the University of Minnesota granted the degree of Bachelor of Arts. Passing straightway into graduate studies, Ranson placed himself under the direction of Professor Donaldson and pursued a course of neurological training that led to the degrees of Master of Science in 1903 and Doctor of Philosophy in 1905. At this juncture Dr. Ranson decided to continue with the regular medical curriculum. To this end he graduated from Rush Medical College in 1907, completed an internship at Cook County Hospital in 1908, and then opened an office for the practice of medicine in Chicago.

But at this very time Dr. A. W. Meyer came to Northwestern University as professor of anatomy and was looking for a well-trained assistant. An arrangement was made by which Dr. Ranson became an associate in anatomy, although continuing to maintain an office and keep noon and evening hours. All thoughts of a clinical career, however, were soon superseded by the more enticing prospects of a full-time academic appointment with higher rank. The opportunity arose through Dr. Meyer's departure for Stanford University after only a 1-year incumbency of the Northwestern chair. Recognizing Dr. Ranson's worth and potentialities, Dr. Meyer recommended him as his eventual successor. This advice the University followed, though it was a bold move and involved promoting a young man over other well-qualified and older teachers in the department. In 1909, Dr. Ranson was promoted to an assistant professorship, and to prepare him further for his duties it was decided that a year of foreign study

would be beneficial. In accordance with this plan he was promoted to an associate professorship and the year 1910-1911 was spent in Professor Wiedersheim's laboratory at Freiburg. This experience was profitable in a general educative way, but the research opportunities were not all that had been hoped for; from it came the conviction that no longer were the continental laboratories superior in their offerings to young Americans over what could be gained at home. Returning from his foreign year Dr. Ranson became in 1911 professor of anatomy and chairman of the Department of Anatomy at Northwestern University. This post he held for 13 years.

In 1924, although the munificent gift of Mrs. Montgomery Ward to Northwestern University Medical School had been announced, Dr. Ranson decided to accept an attractive offer from Washington University to become professor of neuroanatomy and assume charge of the teaching of histology and neuroanatomy in that institution. But less than 4 years later Northwestern was able to counter with a proposal wholly to his liking. The move to St. Louis had been actuated partly by the reduction of teaching duties and a corresponding increase in available research time. Now it was proposed that on his return to Northwestern teaching would be eliminated and all his hours devoted to a research institute, organized according to ideas that had been formulating in his ever-progressive mind. It was a challenge he could not resist and for the next 14 years he gave his all to bring to successful fruition this dream of what he wanted most to do.

Dr. Ranson's lifetime academic labors were devoted to teaching, administration and research. From 1908 to 1915 he taught gross anatomy solely, and during this period received help chiefly from student assistants. Only after the death of Professor Prentiss, in 1915, did he also instruct in neuroanatomy; thereafter this subject came to take more and more of his teaching attention until his formal pedagogical career came to an end in 1928. The teaching of histology at Washington University was but a relatively brief incident in his 20 years

of teaching experience. As a teacher Dr. Ranson was conscientious, informative and sound. His lectures were carefully prepared, skillfully organized and well chosen as to subject matter. His speaking style was matter-of-fact and somewhat lacking in spontaneity and ease. Of this defect he was keenly aware, and although he strove to correct his style he never attained a fluent and dynamic platform delivery. More effective was his individual instruction in the laboratory, where his courteous and sympathetic manner, his eagerness to be of help, and his eminent fairness made a perfect foil to his insistence on high standards of accurate observation and straight thinking. Although the force of his quiet personality and scientific eminence left an indelible imprint on a generation of students who sat under him, far broader in pedagogical influence is his textbook, "The Anatomy of the Nervous System," which has passed through seven editions since 1920. Before its advent there was no suitable student-text in this subject and recourse had to be made to the chapters included in works on gross anatomy. Its preparation was begun after only 2 years of teaching in neuroanatomy, yet it was outlined and elaborated with sureness and speed. When it is realized that there was no previous pattern to aid in formulating a plan of treatment, it is remarkable that this authoritative book, which had to take second place to the encroachments of a heavy teaching schedule and continuous productive research, and was interrupted for many months by a nearly fatal pneumonia, reached so speedy a conclusion. It is a fine tribute to the author's judgment that this pioneer text has stood without fundamental alteration in plan of presentation through numerous editions and still remains the leader in its field.

As an administrator and director of laboratories Dr. Ranson had gifts of a high order. His natural flair for leadership, his deep sense of responsibility, his infectious enthusiasm, his integrity of character, his unswerving reliability, his purity of ideals, his transparent honesty, his innate fairness and his sympathetic friendliness have inspired a multitude of col-

leagues, graduate students and subordinates to enduring sentiments of loyalty, esteem and devotion. He had the rare ability to make the utmost of what was available in facilities and funds. In the early days these were small, yet so judiciously were they turned into productiveness that in retrospect the feat still must command admiration. Later on, in the Institute, this early training continued to pay dividends, and no organization of its kind probably has seen more effective management in terms of cost accounting on the basis of income and output. One objective was ever foremost, and that was efficient production; he drove himself without stint and demanded the same unsparing application from others. Dr. Ranson was a keen judge of human nature and was unusually successful in selecting the proper person for any post, whether it were as a teacher, a research associate, a graduate student or a technical assistant. To this end he spent countless hours of inquiry and sifting of credentials, since in the closely knit organization that he required, compatibility and team-work were as essential as ability. Through his successes in these several directions the reputation and influence of the Institute rapidly gained a world-wide recognition that attracted students from many lands. The number of subsequent teachers, investigators and clinicians whom he trained over a 30-year period is an impressive living monument to his memory. Of the actual scientific grist of his Institute, the fourteen published volumes speak more eloquently than any encomium. High in quality, as in quantity, they represent the yield of what a competent judge has characterized as "one of the most productive schools of neurology that has ever existed."

The dominating interest in Dr. Ranson's intellectual life was research. It began while he was yet an immature college student and grew in intensity with the years. It was his spoken conviction that research brings the highest degree of intellectual exhilaration and satisfaction that the human mind is capable of achieving. It was, therefore, with unconcealed enthusiasm that he welcomed the opportunity to abandon

routine teaching and limit his labors to the field of neurological investigation. So great was his zeal, that he had hoped to continue actively in research even after official retirement would be forced upon him. Dr. Ranson's scientific achievements did not follow the curve that is usually correlated with age. Throughout his life he continually increased in scientific stature and his later years deservedly brought him his greatest fame. He was always eager to make use of new techniques and equally ready to enlist the cooperation of those who had special qualifications beyond his own. A great part of his later successes were due to this ability of devising a careful program of action and then delegating its execution largely to trusted lieutenants. Dr. Ranson was a passionate searcher for truth, wherever it might lead. He was highly critical in evaluating evidence and extremely cautious in drawing conclusions. Nothing could be accepted as the truth until it had been repeatedly checked, thoroughly balanced with controls and all alternative avenues of interpretation by-passed. In this atmosphere of thoroughness, candor was encouraged. Freedom in questioning and criticising was complete. But the subordinate who took on his Chief in argument had to be prepared for a battle royal or be doomed to ignominious defeat, for he was a fair but relentless antagonist. Rather paradoxically this aptitude for informal wit-matching was not carried to the public forum. At scientific meetings he was somewhat diffident and loath to engage in sharp debate, and frequently let his cause suffer by mildness and incompleteness of reply. Fortunately he had fighting disciples, and it was sometimes amusing to see former students rush to his aid with the marshalled rebuttals that he himself could have advanced.

A bibliography of Dr. Ranson's publications contains 211 titles, 147 of which date from 1928. Early morphological interests soon shifted into studies that dealt with functional interpretations of structural backgrounds. Yet even to the end he was ready to engage in long investigations of pure morphology if these were necessary to clear the way or

illuminate dusky functional mechanisms. That he was the outstanding exponent of a definite school of combined neuro-anatomy and neurophysiology is scarcely subject to question. It is not necessary to record beyond mere mention the varied fields of his endeavor. At first these included studies on: nerve degeneration and regeneration; the perfecting of the pyridine-silver staining method; the demonstration of unmyelinated nerve fibers in sensory nerves; their relation to pain sensation; analyses of peripheral visceral nerve distributions and central pathways; and studies on vasomotor centers. Then his attention became directed to the mechanisms involved in postural contractions, and so to spinal reflexes and the regulation of limb reflexes. It was a natural step to proceed to the higher centers, but in order to approach these buried structures he was forced to revive and refine the technique whereby the almost abandoned Horsley-Clark stereotactic apparatus could be made a dependable tool. There followed a rich procession of publications on the midbrain, and especially on the hypothalamus and its varied activities and relations. Other studies of this period dealt with the pathways for pupillary light reflexes, with the cerebellum and, latterly, with the corpus striatum. It is interesting that his last activities were directed toward a re-entry into the problems of nerve regeneration, which would have brought the cycle, by a full turn, back to the field of his doctoral dissertation.

Many honors came to Dr. Ranson. He was a fellow of the American Association for the Advancement of Science, a councillor of the Society of Experimental Biology, and a member of the American Neurological Association, of the American Physiological Society, and of the American Association of Anatomists. In the latter association he served on the executive committee in 1921-1924, was vice-president in 1926-1928, and president in 1938-1940; for some years he also served on its committee on nomenclature. He was a member of the editorial board of the *Archives of Neurology and Psychiatry*. In 1940 he was elected a member of the National Academy

of Sciences. He was a member of the Sigma Xi, Alpha Omega Alpha and Phi Beta Pi scientific and medical fraternities. In 1929 the Northwestern Chapter of the latter established The Stephen Walter Ranson Lectureship in his honor. Requests came to deliver lectures before various learned bodies; among these were: the Weir Mitchell Oration (1934); the Harvey Lecture (1936); the Dunham Lectures (1940); and the Hughlings Jackson Lecture (1941). To him was dedicated the imposing volume on "The Hypothalamus and Central Levels of Autonomic Function," assembled from a symposium held at the 1939 meeting of the Association for Research in Nervous and Mental Disease.

Dr. Ranson was by nature dignified, yet unassuming, and of serious demeanor. He was not interested in social activities of a formal nature, and still less so in promoting himself by such means. Though somewhat reserved before strangers, he enjoyed thoroughly the companionship of well-tried friends and fellow workers; none who knew him intimately could doubt the genuineness of his interest in their welfare and the quiet, yet sincere, cordiality of his greeting and intercourse. As a modest man he disliked bombast and sham, and his keen perception was quick to see through those who were pretentious beyond merit. Although his interests ran deep in certain subjects outside his professional field, and especially so in current affairs, they were not notably wide-ranging. Most of his energy was conserved toward the use of the working day, and his evenings were dedicated largely to the quiet enjoyment of his family. This conservation of strength was partly enforced by a gastric ulcer of long standing which gave great trouble during the last 10 years of his life. In September, 1941 Dr. Ranson suffered a coronary attack from which he slowly made a partial recovery, but a recurrence on August 30, 1942 brought a fatal termination almost instantly. The same malady had caused the death of his father and grandfather. He is survived by his devoted wife and three children. The influence of Dr. Ranson's life and work has left an enduring mark on Northwestern University, on a host of past and present associates and on American anatomy. A great and a good man has passed from among us.

L. B. AREY

GEORGE WASHINGTON CRILE



1864-1943

George Washington Crile was born at Chili, a village in north-central Ohio, November 11, 1864, and died in Cleveland, January 7, 1943. He was graduated in arts in 1884 at what is now Ohio Northern University and in medicine at the Medical Department of Wooster University in Cleveland in 1887.

Dr. Crile was widely known as a skillful surgeon. His teaching career and his interest and work in anatomy are less well known. He began his medical teaching in 1889 as lecturer and demonstrator of histology in the medical school from which he was graduated. A year later physiology was added and from 1893 to 1896 he was Professor of Physiology. After 1896 his teaching was entirely in surgery. He was Professor of Surgery in the same medical college from 1896 to 1900 and was a professor of surgery in the School of Medicine of Western Reserve University from 1900 to 1924, when he resigned to devote himself to other medical interests. He taught in medical schools in Cleveland continuously for 35 years.

He began animal experimentation in physiology in 1893. This led to publication of a book on shock in 1897. His experimental work in physiology brought interest in comparative organology, which he investigated in domestic and feral animals of northern Ohio and in all the types of exotic animals he was able to secure from the local zoological garden. It was this interest that led to his becoming a member of the American Association of Anatomists in 1910.

His work in comparative organology was at first confined to the adrenals and thyroids and later extended to the heart, sympathetic nervous system, and central nervous system.

He, with three medical associates, established the Cleveland Clinic in 1921. In this, at the outset, was a laboratory for animal experimentation and comparative organology, small at first, but later provided with an adequate building and a considerable full time staff of individuals well trained in science.

After his retirement from teaching Dr. Crile began explorations in 1927 to study the action of wild animals in their native habitat and to collect the organs in which he was interested. These expeditions, continued until 1941, extended in North America from the shores of Hudson Bay to Panama, to South America, Central Africa, and Pacific islands. He took with him a trained zoologist, a technician and equipment to set up a laboratory in the field. Animals that were killed were immediately dissected to expose the organs in which he was interested. These were photographed or sketched in situ, then extirpated and accurately weighed in fresh condition and the weights compared with the total body weight. The organs were preserved and sent to the laboratory in Cleveland for further gross and microscopical study. The greater part of the work was on mammals, ranging from small rodents to manatees, whales, and elephants. Some attention was given to reptiles and birds.

Accurate comparative weight determinations in the fresh condition of each of the organs mentioned have been made and compared with the other organs, and with total weight of the individual animals for nearly 4000 sets of specimens. Some of this work has been published. Endowment of the laboratory in Cleveland assures that the study of these extensive collections will continue, with resulting publication.

Dr. Crile was engaged personally on experimental and comparative research for nearly 50 years, from 1893 until a few months before his death, and aided in later years by a staff of workers under his direction. He was the author of

twenty-four published books and over 500 articles in periodical literature. His research in physiology and comparative organology much influenced his surgical work and led to notable contributions to surgical practice and technique.

Dr. Crile was a man of personal charm, with great energy and tireless industry. He was generous in financial contributions to many cultural, medical, and scientific undertakings. He was a member of many medical and scientific societies of the United States and other countries, and received numerous testimonials of high regard of his scientific and surgical work. He was a surgeon in the Spanish American and first World War with foreign service in both, and was a brigadier general in the Reserve Corps from 1921.

FREDERICK C. WAITE

FRANKLIN PARADISE JOHNSON

1888-1943

Franklin P. Johnson was born in Hannibal, Missouri, January 7, 1888, the parental Paradise and Johnson families being of French and English ancestry. Of his Missouri boyhood we have little information, but he sold stereoscopes and worked for a jeweler to support himself at the University of Missouri, where he received the A.B. degree in 1908. As undergraduate he took five medical school courses in anatomy under Prof. C. M. Jackson, with grade A in each of them. Then, when he saw on the bulletin board a notice sent by Prof. Charles S. Minot to his friend Dean Jackson, stating that a Teaching Fellowship in microscopic anatomy was available at the Harvard Medical School, "Johnny" applied and was accepted. He came like a breath of fresh air in a musty laboratory, and the professor, captivated by his buoyant personality, cleared the way for rapid advance. Johnson was athletic —



full of life and energy. At a surprising remark he would turn a back somersault in the air; he had his mandolin for solace; and if the professor on arrival in the morning found his desk chair already occupied and tilted back, he welcomed the nonchalant greeting from his student Fellow. In this country one does not come to attention and salute as the head professor passes by.

In the Harvard laboratory Johnson found an assistant professor who had received a belated assignment to write the chapter on the development of the digestive tract for the Keibel-Mall "Human Embryology." That combined undertaking by German and American authors, promising closer relations than ever with the German scholarship so much admired by all, was an opportunity which could not be declined, though in the time available it could not possibly be met. Hence, as adroitly as possible, the advantages of a study of the transition from the original smooth entodermal lining to the serviceable pits and elevations of the digestive apparatus at birth, were suggested to the newcomer, for whom the head professor had proposed a program of his own — the blood supply and vascular relations of the suprarenal gland. Johnson chose the digestive tube, and had a paper on that subject ready for the Boston meeting of the Anatomists, in December, 1909, when he was elected member of the Association. Ultimately he made upwards of fifty wax reconstructions of the intestinal mucosa. Some of them were described in his first publication (1910), whereupon he received the Harvard A.M. and was appointed instructor in histology and embryology.

The summer of 1911 was spent at Keibel's Institute in Freiburg. Johnson arrived there after a two-weeks' tour of Italy, reporting that he should always long for the time when he could return; but he had business elsewhere. Professor Keibel received him most hospitably in his laboratory, where he was soon at home. Leaving some tissue in fluid that he wished stirred, he placed it in a passageway, with a label — "Shake it up." The professor himself, pausing to inspect the outfit,

soberly complied with directions and passed on. On the visit of Dr. and Mrs. Clark, Johnson was delegated to show them the "Institut." He met Gaupp — Wiedersheim was absent — and found Ranson and Evans there, all sending greetings to Minot. Johnson added that, to his surprise, the Keibel collection of sectioned embryos was very poor in comparison with Minot's, referring to a technique which, as further developed by his friend Heuser, was to become unsurpassed anywhere.

On returning to Boston, the intestinal researches were continued to the colon. Johnson discovered for himself, and pictured effectively, the virtual obliteration of glands and villi on distention of the small intestine of the newborn guinea-pig, and their return to normal shapes when pressure is reduced. This property is unusually well-developed in the guinea-pig, where we learned that Heitzmann had noted it in 1868. With all this work as a thesis (published in two papers in 1913) Johnson obtained his Harvard doctorate in Philosophy (June, 1912). Not always did the assistant professor's advice outweigh that of his superior, for Johnson had followed the latter in seeking his degree in philosophy rather than in medicine. Looking back to this time Dr. Johnson wrote (from Johns Hopkins, June 22, 1919):

"From my own experience I believe the M.D. training is more broadening and possibly more fitting for one going into the teaching of anatomy. But there are things to be said against the M.D. training as it is now given . . . In my own class here . . . at least fifty per cent of the medical students show evidence of originality if they were given the chance. But no — the ablest students dig and grind away . . . Perhaps the greatest drawing card for the Ph.D. is that one can make a livelihood while working for the degree. This to me was a very important consideration."

Dr. Johnson was immediately recalled to Missouri as assistant professor of anatomy (1912), and in the following year, when Professor Jackson accepted a call to Minnesota, he was promoted to associate professor, and became, *pro tempore*,

head of the department. In Boston he had met a talented student of the Museum School of Art, Miss Annette Gavett, and their wedding, planned for 1912, had been postponed because of nervous illness. It took place in August, 1913; and full of pranks as of old, he was deliberately late at his wedding to show his unconcern. But the bride was later yet, and his best man anxiously sought her out. Their years in Missouri were happy and busy. A house was built. Researches on the intestinal tube were completed with a much needed account of the development of the rectum and anus (1914, 57 pages), and a casual report of atresia ani in a 26-mm. embryo (1914). The value of these digestive tube studies is suggested by the fact that five of Dr. Johnson's figures — perhaps more than those of any other American anatomist — have been reproduced in Von Möllendorff's splended "Handbuch."

After Dr. Clark had accepted the position of professor of anatomy in Missouri (1914), he came to know "how able and experienced Dr. Johnson was — a good teacher, a fine investigator, and an excellent administrator." The work of instruction was divided; each had his own student or graduate assistants. With their aid Johnson completed a monograph on a human embryo of twenty-four pairs of somites, received from a Missouri physician (Carnegie Contributions, 1917). He brought back into general use Wepfer's neglected method (dating from 1664) of shaking apart the pig's liver into its constituent lobules (1918); and he studied their development with clearer results than Mall had been able to attain (1919). But the war was then on, practitioners were needed, salary distinctions were invidious, and Dr. Johnson began to think, in the phrase of John Brown, that *Anatomy is statical, it has no feet*. In 1918, he enrolled at Johns Hopkins as a student of medicine in advanced standing. He was not alone in that procedure, for his friend, Roy Hoskins, Ph.D. (Harv.), professor of physiology at Northwestern, had decided to take the same course at the same time. On occasions the erudition of these students astonished their teachers.

In Baltimore, Johnson completed what is perhaps his finest anatomical work — the models of the later development of the urethra in the male (*Journal of Urology*, 1920). A parallel study, "The homologue of the prostate in the female" (*ibid.*, 1922), was in progress. He did much of the modeling at the Carnegie Laboratory, where Dr. Streeter was impressed with his fine sense and appreciation of form, and his great energy and abilities. The models are in no way inferior to the superb Keibel series, which indeed they continue to a later and most interesting stage.

In 1919-1920, the University of Missouri promoted him to a full professorship in anatomy, but it was too late. His M.D. was conferred on June 15, 1920. "Tomorrow," he writes on the fifteenth, "I am going fishing down on the Severn," and then to the New Haven Hospital as intern with Dr. Flint. There, in a staff football game, he fractured his patella, and after 7 weeks' quiescence, was hobbling about with cane and crutches — this being but one of several surgical incidents due to his fondness for heavy gymnastics. Later in that year came family disaster in the death of his wife after prolonged hospitalization (August 23, 1921). He had then returned to Baltimore. Resolutely, on Labor Day, he began his service as first assistant resident in urology at the Brady Institute of the Johns Hopkins Hospital. In that specialty he had found his ultimate calling, and Dr. Young invited him to remain on his staff. But Dr. Johnson had other plans. For a place to practice urology, he considered the various States, choosing Oregon, and Portland, because there, it seemed to him, was the best fishing near a great city. On a high hill, in sight of Mt. Hood, he could build a "little house amongst the big trees."

In September, 1923, he married Miss Juliette Omohundro, of Virginia; and in October they were established in Portland. "I am settled at last," he writes, "have a suite of beautiful offices, open for business eight days now, without being disturbed by a single patient." Thus began his successful practice. He had time to collaborate with Dr. Hugh H. Young in

writing his authoritative "Practice of Urology" (2 vols., 1926). Since 1929, Dr. Johnson has been assistant clinical professor of urology in the University of Oregon. In 1933, at the Cincinnati meeting of the Anatomists, he described isolated tubules of the human testis, which he had skillfully dissected, as showing loops and arcades, with a few blind ends and diverticula. Thus (1911) he supplemented Bremer's study of the development of the remarkable arcades by showing conclusively their arrangement in the adult. He left the session early, by airplane, to see some new operative technique. For the 9th ed. of Morris' Anatomy, published in 1933, he wrote the section on the urogenital system (revised in the 10th ed., 1942), and he contributed also to Nelson's Loose-Leaf Surgery (vol. 6, 1940). In 1935 he demonstrated to the American Urological Association in San Francisco a new type of snare, which he had devised, for removing ureteral calculi. The Johnson "basket" is now the most used of all snares for that purpose. A Boston surgeon remarks, "We have had very good success with it, which is more than we can say of any other device that has come to our notice." Last June Dr. Johnson met with the Urologists in New York, full of his usual zest; but he was aware of a cardiac lesion, and said something to a friend about "the old pump." Two weeks before his death, which occurred on February 12, 1943, he collapsed on the way home from his office. He is survived by his wife and their three daughters, who know that though his interests were wide and full, his family came first.

In many ways, as we like to think, Franklin Johnson was a typical American anatomist. In his profession he had friends from the Atlantic to the Pacific; and he repaid with compound interest all that he owed to the universities with which he was connected — Harvard, Missouri, Johns Hopkins, and Oregon. This is an indaequate record, but one of sincere admiration.

April 20, 1943

FREDERIC T. LEWIS

ELIOT R. CLARK

RICHARD E. SCAMMON

ANATOMICAL PUBLICATIONS BY FRANKLIN P. JOHNSON

- 1910 The development of the mucous membrane of the oesophagus, stomach and small intestine in the human embryo. *Am. J. Anat.*, vol. 10, pp. 521-561.
- 1913 The development of the mucous membrane of the large intestine and vermiform process in the human embryo. *Am. J. Anat.*, vol. 14, pp. 187-233.
- 1913 The effects of distention of the intestine upon the shape of villi and glands. *Am. J. Anat.*, vol. 14, pp. 235-250.
- 1914 The development of the rectum in the human embryo. *Am. J. Anat.*, vol. 16, pp. 1-57.
- 1914 A case of atresia ani in a human embryo of 26 mm. *Anat. Rec.*, vol. 8, pp. 349-353.
- 1916 Notes on the neuromeres of the brain and spinal cord. *Anat. Rec.*, vol. 10, pp. 209-210.
- 1917 A human embryo of twenty-four pairs of somites. *Carnegie Contr. to Embr.*, vol. 6, no. 19, pp. 125-168.
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- 1919 The development of the lobule of the pig's liver. *Am. J. Anat.*, vol. 25, pp. 299-331.
- 1920 The later development of the urethra in the male. *J. of Urology*, vol. 4, pp. 447-501.
- 1922 The homologue of the prostate in the female. *J. of Urology*, vol. 8, pp. 13-33.
- 1923 Diverticula and cysts of the urethra. *J. of Urology*, vol. 10, pp. 295-310.
- 1926 *Young's Practice of Urology*. "By Hugh H. Young and David M. Davis with the collaboration of Franklin P. Johnson." Philadelphia, vol. 1, pp. 1-746; vol. 2, pp. 1-738.
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- 1933 *Urogenital System*. Morris' *Human Anatomy*, 9th ed., Philadelphia, pp. 1347-1411; 10th ed., 1942, pp. 1421-1488.
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- 1940 *Surgery of the urethra and penis*. Nelson Loose-Leaf Surgery, vol. 6. New York, pp. 1-98 B.
- 1943 Compromise closure of bladder in suprapubic prostatectomy. *J. of Urology*, vol. 49, pp. 377-381.

HENRY FRANCIS NACHTRIEB

1857-1942

Henry Francis Nachtrieb, Professor Emeritus of Animal Biology at the University of Minnesota, died at his home in Berkeley, California on July 17, 1942, at the age of 86. He



is survived by his wife and daughter. He received the B.S. degree from the University of Minnesota in 1882 and did graduate work at Johns Hopkins University during the following 2 years. He returned to the University of Minnesota in 1885 as assistant and became head of the Department of Animal Biology in 1887, holding this position until his retirement in 1925.

Professor Nachtrieb directed the Zoological Division of the Geological and Natural History Survey of Minnesota and was editor of its publications. His chief interest was in the leeches and fishes of the state, especially the spoon bill sturgeon, *Polyodon spathula*. Much of the survey work was done in the "Megalops," a floating laboratory which he designed for this purpose.

The Department of Animal Biology was established and developed under his direction, and the modern building completed in 1915 and now occupied by the Department of Zoology was constructed according to his plans. The development of the splendid zoological library was due largely to his efforts and continued interest. The large collection of zoological and anatomical journals which in the early days he was able to keep in the departmental library provided a constant stimulus for the research work of the members of his staff. Under his administration the department was founded and developed along broad university lines, and facilities for advanced work and research were provided as rapidly as funds would permit. Each member of his department was encouraged to develop the field of his major interest.

Building up the department to a high state of efficiency within relatively few years was Nachtrieb's chief contribution. His interests were broad and were not confined to zoology. Anatomy was one of his fields of interest and he attended many meetings of the Association. In his later years he became interested in animal genetics and introduced the first course in the subject at the University of Minnesota.

E. A. BOYDEN
HAL DOWNEY
C. M. JACKSON

HENRY ERDMANN RADASCH



1874-1942

Henry Erdmann Radasch, Emeritus Professor of Histology and Embryology of the Daniel Baugh Institute of Anatomy of the Jefferson Medical College, died November 29, 1942, in Philadelphia, Pennsylvania.

Dr. Radasch was born in Keokuk, Iowa, May 7, 1874. He was graduated from the State University of Iowa in 1895, receiving the B.S. degree. He served as Fellow in Chemistry and Assistant in the Chemical Laboratory at his Alma Mater while studying for the M.Sc. degree, which was conferred in 1897. As Lecturer in Inorganic Chemistry and Director of the Chemical Laboratory, College of Physicians and Surgeons, Keokuk, Iowa, he had his initial contacts with the field of medicine during the academic year, 1897. He matriculated at the Jefferson Medical College in 1898, gaining the M.D. degree in 1901. During the progress of his medical course he assisted in the Laboratory of Histology.

Following his graduation, Dr. Radasch served as Assistant Demonstrator of Anatomy and Demonstrator of Visceral Anatomy in addition to his duties in the histology laboratory. He became Assistant Professor of Histology and Embryology in 1911. During the academic year 1912-1913, he was Acting Director of the Daniel Baugh Institute of Anatomy. He was advanced to an Associate Professorship in Histology and Embryology in 1918 and to a full professorship in 1921. Having reached the age of retirement Dr. Radasch relinquished his teaching responsibilities in 1942, and in recognition of his devoted and valued service the Board of Trustees of the Jefferson Medical College appointed him Emeritus Professor of Histology and Embryology.

In addition to his duties at the Jefferson Medical College, Dr. Radasch, in the early years of his medical career, was associated with other institutions as well. He studied at the University of Leipsic, 1903. He was instructor in Histology

at the Pennsylvania College of Dental Surgery from 1906 to 1909 and Adjunct Professor of Physiology, Pathology, and Bacteriology from 1908 to 1909. During a period of 6 years, 1913 to 1919, he was Instructor in Anatomy in the Pennsylvania Academy of Fine Arts.

Despite his extremely heavy teaching schedule, Dr. Radasch found time to devote to original investigation and writing. Among his scientific contributions are numerous communications on anatomical and allied subjects, including significant studies on the composition and senility of bone, the histology of the stomach, superfetation, teratology in domestic animals, achondroplasia, muscle anomalies, and osteogenesis imperfecta. He was the author of "A Manual of Anatomy," "A Compend of Histology," and "A Manual of Histology." Further, he assisted Dr. E. A. Spitzka in several revisions of "Gray's Anatomy" and contributed a number of articles to the "Reference Handbook of Medical Sciences."

Dr. Radasch was a member of the American Association of Anatomists, the Philadelphia Academy of Natural Sciences, The Medical Club of Philadelphia, Nu Sigma Nu fraternity, the Alpha Omega Alpha Honorary Medical Fraternity, and other organizations. He served as treasurer and as a member of the Executive Committee of the Anatomical Board of Pennsylvania for many years. He was a philatelist of distinction, his collections and exhibits being known the world over.

Dr. Radasch gave freely of himself in all his endeavors; his was a truly dynamic personality, friendly and kindly. His loyalty was outstanding, his sincerity unquestioned, and his frankness refreshing. Without doubt Dr. Radasch's greatest contribution has been the remarkable service rendered his beloved Jefferson Medical College in giving 44 years of his life to the teaching and training of medical students. During his tenure approximately 7000 medical men were taught by him. All came to love him. His friends in the American Association of Anatomists, his colleagues, and his former students, alike, grieve his passing.

A. J. RAMSAY

N. A. MICHELS

J. PARSONS SCHAEFFER

PROCEEDINGS OF THE AMERICAN ASSOCIATION OF ANATOMISTS

1943

In deference to the desire to restrict civilian travel, the Executive Committee regretfully voted to postpone the Fifty-ninth Meeting of the Association, which had been scheduled to be held in Montreal on April 21, 22 and 23, 1943, at the invitation of McGill University. It was decided, however, to proceed with all feasible Association activities — to publish abstracts of papers; to receive nominations for membership; to authorize the holding of “informal Sectional Meetings,” “limited to local inhabitants so that travel and load on hotel accommodations will not be increased”; and to publish the annual Proceedings, “which may include brief reports of Sectional Meetings.”

On February third there occurred a serious blow to the Association in the death of our President, Edgar Allen. The Secretary-Treasurer, Dr. Swett, turned at once to the First Vice-President, J. Parsons Schaeffer, who carried on as Acting President. Scarcely had this arrangement been completed, when, on February tenth, the Association suffered a second devastating blow in the death of our Secretary-Treasurer, Dr. Francis H. Swett. With no provision in the Constitution covering such an emergency, but relying upon Article III, which states: “The management of the affairs of the Association shall be delegated to an Executive Committee,” Dr. Schaeffer requested the Nominating Committee to propose

a substitute ad interim — to serve until the next meeting of the Association. The Committee proposed the name of Dr. Eliot R. Clark, who had been Dr. Swett's immediate predecessor in the office. By mail vote the Executive Committee endorsed the selection of the Nominating Committee, and on March 5, 1943 Dr. Clark became Acting Secretary-Treasurer. Fortunately, before his death, Dr. Swett had received, censored and sent to The Wistar Institute for printing, titles and abstracts for the Abstract number of The Anatomical Record. He had also received, and investigated the credentials of, nominees for membership. With his striking originality, high standards and common sense he had started his term as Secretary-Treasurer of the Association in such a manner that it is clear that the Association has suffered a substantial loss. He would have injected new life into it, especially while working in conjunction with our able and greatly lamented President, Edgar Allen.

Term of office during the "interim." The Nominating Committee consisting of H. Cummins, Chairman, K. E. Mason and W. F. Windle submitted, for members of the Executive Committee for 4 years, Prof. William Bloom and William W. Gruelich. The Executive Committee voted that "these names will be presented whenever the next annual meeting may be held — the time between the 1942 meeting and that date being considered an interim period, counting as one year, with respect to tenure of officers and members of the Executive Committee." This ruling is interpreted by the Acting Secretary as applying also to elected representatives, except the representative to the National Research Council (see later).

On March 17, 1943 the Acting Secretary-Treasurer sent a report to the members of the Executive Committee, with a request for a mail vote on eleven items. The matters presented were as follows:

1. (a) *Financial statement for the year 1942*

Balance on hand in checking account Dec. 31, 1941 (last audit)	\$1,608.45	
Special savings account of former Treasurer	712.24	
Bank balance received from former Treasurer	804.23	
Receipts from dues	1,334.13	
One dues item entered twice in cash book because first check was returned by bank	2.00	
Total credits		\$4,461.05
<i>Expenditures</i>		
Miscellaneous postage, supplies, printing, programs 58th meeting, addressograph changes, etc.	\$250.60	
Travel expenses — Secretary	13.95	
Local Committee 58th meeting	250.00	
Wistar Institute		
Abstracts and postage	435.72	
Proceedings and postage	162.24	
Dues check returned for signature	2.00	
Seeman Printery Inc.		
Statements and Stationery	75.25	
Series F. War Bond	740.00	
Transfer of balance of new Treasurer	804.23	
Total expenditures		\$2,733.99
		\$1,727.06
<i>Assets</i>		
Cash on hand Citizens National Bank, Durham, N. C. Dec. 31, 1942	\$1,727.06	
War Bond Defense Series F.	740.00	
Total assets		\$2,467.06

1. (b) Report of appointment by Acting President J. P. Schaeffer of an Auditing Committee consisting of W. H. F. Addison, Chairman, and E. J. Farris, who on March 15, 1943 submitted the following report:

March 15, 1943

We have examined the accounts of the American Association of Anatomists for the year 1942 and report as follows:

The records show the assets of the Association to be divided into two categories, "checking" account and "savings" account.

Checking Account

January 1, 1942–May 5, 1942, E. R. Clark, Treasurer: Receipted bills, checks drawn, and deposit slips agree with bank statements. The balance in bank May 5 was \$804.23. This balance was transferred by Cashier's check of First National Bank of Philadelphia (on a "counter" check recorded as no. 80) to Francis H. Swett, Durham, N. C.

This amount is recorded as deposited in the Citizens National Bank of Durham, N. C., May 9, 1942.

May 5, 1942–December 31, 1942, Francis H. Swett, Treasurer: Receipted bills, checks drawn, and deposit slips agree with bank statements. The balance in bank December 31 was \$1,727.06.

Savings Account

From January 1 to May 4, 1942, this account was in the Morris Plan Bank of Philadelphia. The bank book shows an increase from \$703.45, by addition of interest of \$8.79, to \$712.24. On May 4 the account was closed and the balance of \$712.24 transferred to F. H. Swett and deposited by him in the checking account on May 9, 1942. On May 26 Dr. Swett purchased a War Defense Bond, Series F, M 226397 F \$740.00, registered in the name of the American Association of Anatomists. Thus the savings account increased during the year by \$36.55, of which interest accounted for \$8.79, while the remainder, \$27.76, represented funds transferred from the checking account. The bond is now in Dr. Clark's care, deposited in a safe-deposit box at the First National Bank, Centennial Branch, 32nd and Market Sts., Phila., Pa.

We have further, at the request of Dr. E. R. Clark, examined the accounts from January 1, 1943 to March 1, 1943, and find receipted bills, deposits and bank statements in agreement, with a balance, on March 1, 1943, of \$1,747.28 in the Association account in the Citizens National Bank, Durham, N. C.

Signed:

W. H. F. ADDISON
E. J. FARRIS

2. Report that President Allen appointed E. de Robertis of Buenos Aires to represent the Association at the Conferencia Nacional de Anatomia normal y Patologia, in Cordoba (October, 1942) — that an acknowledgment of the appointment but no report had been received.

3. Report that requests for remission of dues had been received by five members, with recommendation that they be granted.

4. Resignation of two members, R. D. Baker and R. S. Stone.

5. Report, and recommendation, of suggestion that dues be remitted to members of the Association in the armed services.

6. Suggestion that for 1943 dues be set at \$1.50, with the explanation that annual expenses with meeting total about \$1200; without meeting, between \$800 and \$900; and that present paying membership is about 683.

7. Report of requests for reinstatement to membership, upon payment of back dues, by R. L. Carpenter and J. B. Goldsmith.

8. Report of the very great loss suffered by the Association since the last meeting through the death of seven members, with request for authorization to print in the Proceedings memorials to all seven, written by committees appointed by Acting President Schaeffer. The memoirs will be found at the beginning of these Proceedings; the names are as follows:

EDGAR ALLEN, born May 2, 1892, died February 3, 1943. Elected to membership in 1921. Professor of Anatomy, Yale University. Vice-President, 1930 to 1932; President, 1942-.

GEORGE CRILE, born November 11, 1864, died January 7, 1943. Elected to membership in 1910. Formerly Professor of Surgery, Western Reserve University.

FRANKLIN P. JOHNSON, born January 7, 1888, died February 12, 1943. Associate Clinical Professor of Urology, University of Oregon; formerly Professor of Anatomy, University of Missouri.

HENRY F. NACHTRIEB, born March 11, 1857, died July 17, 1943. Professor Emeritus, Animal Biology, University of Minnesota.

HENRY ERDMANN RADASCH, born May 7, 1874, died November 29, 1942. Professor Emeritus of Histology and Embryology, Jefferson Medical College.

STEPHEN W. RANSON, born August 28, 1880, died August 30, 1942. Professor and Director, Neurological Institute, Northwestern University Medical School. Member of Executive Committee, 1921-1924; Vice-President, 1926-1928; President, 1938-1940.

FRANCIS HUNTINGTON SWETT, born November 13, 1893, died February 10, 1943. Professor of Anatomy, Duke University School of Medicine. Secretary-Treasurer, 1942-.

9. Report that since E. R. Clark had served as representative to the National Research Council for 3 years, and since the Council places 3 years as the limit of service for any individual, Dr. George L. Streeter be appointed to this position for a 3-year term.

10. Report that two Sectional Meetings, as authorized by the Executive Committee, had been held — in Chicago and Philadelphia (full report elsewhere) and suggestion that the Secretary frame a statement regarding regulations for such future meetings.

11. Report on nominees for membership. The names of twenty nominees were sent to the members of the Executive Committee. Three were questioned and they, together with ten questioned by Dr. Swett, were held in abeyance until the next meeting of the Executive Committee, when their qualifications can be discussed. Seventeen were endorsed by the Committee. As stated in the announcement concerning the annual meeting, "they will be considered as *Provisional Members* until the next meeting, before which time they cannot be voted upon in accordance with Article V, Section 1 of the Constitution of the Association." The names of the new *Provisional Members* are as follows, the names in parentheses being those of the sponsors:

- ALDEN, ROLAND H., B.A., Ph.D., Instructor in Anatomy, *University of Tennessee, Memphis, Tenn.* (K. B. Corbin, J. S. Nicholas.)
- BAKER, BURTON LOWELL, M.S., Ph.D., Instructor in Anatomy, *University of Michigan, Ann Arbor, Mich.* (P. E. Smith, B. M. Patten.)
- BARDEN, ROBERT BRADSHAW, A.B., Ph.D., Instructor in Zoology, *Cornell University, Ithaca, N. Y.* (H. B. Adelmann, J. W. Papez.)
- BRADLEY, E. MORTON, B.A., Ph.D., Assistant Professor of Gross Anatomy, *University of Georgia School of Medicine, Augusta, Ga.* (W. F. Alexander, J. Krafka, Jr.)
- BRUESCH, SIMON RULIN, A.B., M.S., M.B., Ph.D., M.D., Instructor in Anatomy, *University of Tennessee College of Medicine, Memphis, Tenn.* (K. B. Corbin, L. B. Arey.)
- HARD, WALTER LEON, A.B., Ph.D., Assistant Professor of Histology, *University of Maryland School of Medicine, Baltimore, Md.* (C. L. Davis, F. H. J. Figge.)
- JAILER, JOSEPH WILLIAM, B.S., M.S., Ph.D., M.D., Research Assistant in Anatomy, *College of Physicians and Surgeons, Columbia University, New York City.* (P. E. Smith, E. T. Engle.)

- LLOYD, RUTH SMITH, A.B., M.S., Ph.D., Instructor in Anatomy, *Howard University, Washington, D. C.* (R. L. McKinney, W. M. Cobb.)
- MASSON, GEORGES M. C., D. V. M., Ph.D., Research Associate, McGill University, Montreal, Canada. (C. P. Martin, H. Selye.)
- NOBACK, CHARLES ROBERT, B.S., M.S., Ph.D., Assistant Professor of Microscopic Anatomy, *University of Georgia School of Medicine, Augusta, Ga.* (G. L. Kelly, L. Allen.)
- PEELE, TALMAGE LEE, A.B., M.D., Associate in Anatomy, *Duke University Medical School, Durham, N. C.* (F. H. Swett, D. C. Hetherington.)
- POMERAT, GERARD ROLAND, A.B., A.M., Ph.D., Instructor in Biology, *Harvard University, Cambridge, Mass.* (A. B. Dawson, F. L. Hisaw.)
- SATTLEB, DWIGHT G., M.D., Instructor in Anatomy, *State University of Iowa, College of Medicine, Iowa City, Ia.* (W. R. Ingram, E. MacEwen.)
- SAUNDERS, JOHN S. DEC. M., M.B., Ch.B., F.R.C.S., Professor of Anatomy, Chairman of Division of Anatomy, *University of California, Berkeley, Calif.* (R. O. Moody, W. R. Lyons.)
- SCHADEWALD, MELVIN, B.S., M.S., Ph.D., Instructor in Anatomy, *Medical College of the State of South Carolina, Charleston, S. C.* (A. M. Lassek, A. T. Rasmussen.)
- WATERMAN, ALLYN J., A.B., A.M., Ph.D., Associate Professor of Biology, *Williams College, Williamstown, Mass.* (R. Rugh, L. Hoadley.)
- WIERDA, JAY L., Ph.D., Assistant Professor of Anatomy, *School of Medicine, University of Pennsylvania, Philadelphia, Pa.* (E. R. Clark, R. G. Williams.)

Meeting or Meetings in 1944. No decision has been reached regarding a general meeting in 1944, and, in view of the rapidly changing conditions, none will be made until the Fall of 1943, when the possibilities will be canvassed. There is a divergence of opinion among our members regarding the desirability of planning a general meeting during the war. Some maintain that the authorities in Washington are not unanimously opposed; that such a meeting would aid the "war effort"; that most of the general objections to such meetings could be met, by avoiding the busy Easter week-end, by meeting in a "non-defense" city away from the Atlantic sea-board, and by holding the meetings on Friday, Saturday and Monday, thus avoiding travel on the days Friday through Monday; that our group is a relatively small one — etc. It is of interest that President Allen favored the Montreal meeting this year, and Prof. Martin of McGill wrote: "I would like to point out that all the societies of England despite bombings and everything else maintain their scientific meetings, and I think it would be

a great disaster to allow any excuse to suppress completely our scientific and intellectual activities."

During the preparation of these Proceedings, announcement was received from the Secretary, Prof. A. J. E. Cave, of a meeting of the Anatomical Society of Great Britain and Ireland, to be held May 21 and 22, 1943; twenty papers and nine demonstrations were scheduled.

In case a general meeting is again postponed or omitted, the Executive Committee will probably repeat the authorization of local Sectional Meetings. Obviously such meetings, if held under the name of the "American Association of Anatomists" should maintain the established standards and procedures of the Association. This, however, should apply rather to the quality of papers presented, than to the specific regulations regarding number of presentations permitted per member, since restrictions in the latter have been designed to keep within reasonable bounds the number of platform papers — a problem which does not apply to the much smaller Sectional Meetings. This year the Committee which arranged the Philadelphia Sectional Meeting preferred to have titles and abstracts of all papers submitted in the regular way to the Secretary of the Association; while the Chicago Sectional Meeting Committee accepted titles directly. The chief difference in the results lies in the fact that abstracts were printed of all except one of the Philadelphia papers, but of less than one-half of the Chicago papers. Possibly provision should be made for printing such abstracts in the Proceedings, but it would seem simpler to handle them in the regular way. Furthermore, the officers of the Association can more effectively protect local committees from occasional under-standard offerings.

The Acting Secretary therefore suggests that those making tentative plans for 1944 Sectional Meetings consider seriously the advantages of operating through the National Secretarial route. Provision will be made for titles and abstracts of demonstrations and motion pictures, as well as of papers from platform and by title, and restrictions regarding number

of papers to be given or sponsored by a single member will undoubtedly be relaxed.

The 1943 Sectional Meetings

The following statement appeared in the notice concerning the Annual Meeting, which was sent to all members in January by the Secretary, F. H. Swett:

"It has been suggested that two or more schools might wish to promote, and run, informal Sectional Meetings at the time of the postponed annual meeting. If so, it appears that they should be limited to local inhabitants so that travel and load on hotel accommodations will not be increased. . . . Brief reports of such meetings can be published in the Proceedings."

In response to this suggestion, two Sectional Meetings were organized, one in Chicago and the second in Philadelphia.

The Chicago "Sectional" Meeting was held at the University of Illinois College of Medicine, Chicago, on Friday, April 23 and Saturday, April 24. Since invitations to take part in the meeting were sent to places outside the Chicago area as far away as Cleveland, Louisville, St. Louis and Iowa City, the Chicago meeting should be labelled a "Regional" rather than a "Sectional" Meeting. The Governing Committee was made up of representatives of the various medical schools in Chicago, with Dr. L. B. Arey, Chairman, and Dr. G. Von Bonin, Secretary. Titles of papers and demonstrations were received by the Committee, with March 15th as the final date. Of the 33 papers submitted, titles and abstracts of 13 were sent to the Secretary of the Association, and are printed in the Abstract number of the Anatomical Record (vol. 85, no. 3, 1943). The remaining 20 titles are without abstracts. There were 4 motion pictures and 9 demonstrations, the former shown in an evening session and the latter throughout the meeting. A memoir of former President S. W. Ranson was read by Leslie B. Arey. Social arrangements included luncheon, tea and dinner—all held on Friday. A resolution of thanks to Professor Kampmeier and the University of Illinois College of Medicine was adopted, and there was universal agreement that the meeting was a great success.

The program was as follows:

Friday, April 23, 10:00 a. m.

- Deep communications of the renal veins. Barry J. Anson, Earl W. Cauldwell and James W. Pick, Department of Anatomy, Northwestern University Medical School.
- Postrenal duplication of the inferior vena cava. Earl W. Cauldwell, Department of Anatomy, Northwestern University Medical School. (Introduced by Barry J. Anson.)
- Structural alterations in the nervous system resulting from anoxia at birth. W. F. Windle and R. F. Becker, Institute of Neurology and Department of Anatomy, Northwestern University Medical School.
- On the control of the position and movements of the human mandible. Allan G. Brodie, Department of Orthodontia, College of Dentistry, University of Illinois.
- Sensory type neurons in the hypoglossal nerve. Anthony A. Pearson, Department of Anatomy, Loyola University School of Medicine.
- Correlation between sex and chemical composition of the brain of white rats. Arthur Weil, Institute of Neurology, Northwestern University Medical School. (Introduced by W. F. Windle.)
- The ascending auditory pathway in the brain stem of the monkey. W. T. Barnes and H. W. Magoun, Institute of Neurology, Northwestern University Medical School.
- The ameloblasts in tooth development with respect to the cellular problems of multiplication, differentiation and change in function. F. Wassermann, Department of Anatomy, University of Chicago.
- Intracentral and peripheral factors in the differentiation of motor neurons in transplanted lumbo-sacral spinal cords of chick embryos. Elmer D. Bueker, Department of Anatomy, The Chicago Medical School. (Introduced by John J. Sheinin.)
- Stephen Walter Ranson, 1880-1942. In Memoriam. L. B. Arey, Department of Anatomy, Northwestern University Medical School.

Friday, April 23, 2:00 p. m.

- An analysis of the role of magnesium sulphate in evacuation of the biliary tract. E. A. Boyden, George S. Bergh, John A. Layne, Department of Anatomy, University of Minnesota.
- The antidiuretic potency of the neurohypophysis of the cat after stalk section. Kendrick Hare and Donald M. Phillips, Department of Anatomy, State University of Iowa, College of Medicine.
- The stria terminalis longitudinal association bundle and precommissural fornix. C. A. Fox, Department of Anatomy, Marquette University and Institute of Neurology, Northwestern University Medical School.
- Early development of the human corpus luteum. William W. Greulich, Western Reserve University School of Medicine.

Further studies on adrenal innervation. Transplantation as a method in determining the distribution of nerves to the adrenal gland. William E. MacFarland, Department of Anatomy, The Chicago Medical School. (Introduced by John J. Sheinin.)

Observations on the polyuria produced by desoxycorticosterone acetate. C. A. Winter and W. R. Ingram, Department of Anatomy, The State University of Iowa, College of Medicine.

An experimental analysis of the inferior mesenteric plexus. Albert J. Harris, St. Louis University School of Medicine. (Introduced by Albert Kuntz.)

The vaginal smear of the rat: Some new questions partially answered. Carl G. Hartman, George Svihla, R. C. Melick, Department of Zoology, University of Illinois.

A comparison of certain mammalian midbrain centers. Elizabeth Crosby and Russell T. Woodburne, Department of Anatomy, University of Michigan.

Morphologic effects of prostigmine, quinidine and curare on the secretion mechanism of motor end plates. Eben J. Carey, Leo C. Massopust, Walter Zeit, John T. Schmitz, James T. Keyes, Department of Anatomy, Marquette University.

Morphologic effects of fatigue and anoxia on the secretion mechanism of motor end plates. Eben J. Carey, Leo C. Massopust, Walter Zeit, John T. Schmitz, James T. Keyes, Department of Anatomy, Marquette University.

At this session W. F. Windle showed lantern slides illustrating S. I. Kornhauser's quadruple stain. (The slides were also demonstrated.)

Friday, April 23, 8:30 p. m.

Motion pictures:

Observations on the minute vessels and lymphatic capillaries in the wing membrane of living bats. Paul A. Nicoll, Department of Physiology, Indiana University Medical School. (Introduced by R. L. Jones.)

Dissection of the cranial cavity for use in teaching gross anatomy. David S. Jones, Loyola University School of Medicine.

Developmental anomalies of the face and mouth. J. J. McDonald, Department of Anatomy, Northwestern University Medical School. (Introduced by L. B. Arey.)

Brain lesions and affective behavior in cats. Max D. Wheatley, Department of Anatomy, The State University of Iowa, College of Medicine. (Introduced by W. R. Ingram.)

Saturday, April 24, 9:00 a. m.

Regenerative processes induced by gonadotropic hormones in irradiated testes of the albino rat. J. M. Essenberg and Emanuele Momigliano, Loyola University School of Medicine.

On the "crypts" of the human pharyngeal tonsil. L. B. Arey, Anatomy Department of Northwestern University Medical School.

Phagocytosis of colloidal thorium dioxide. O. A. Mortensen, Department of Anatomy, University of Wisconsin.

- The cytology of the human trophoblast in relation to hormone production. Burton L. Baker, S. J. Hook, A. E. Severinghaus, Departments of Anatomy, University of Michigan and Columbia University.
- The mechanism of nasopharyngeal occlusion. Leon H. Strong, Department of Anatomy, University of Michigan.
- The role of sebaceous glands in absorption of lipid-soluble carcinogens applied to skin. William L. Simpson and William Cramer, The Barnard Free Skin and Cancer Hospital.
- The type of paralysis following section of the basis pedunculi and dorsal lateral chordotomy in *Macaca mulatta* monkeys. B. W. Cannon, L. E. Beaton, S. W. Ranson, Jr., H. W. Magoun, Institute of Neurology, Northwestern University Medical School.
- Non-production of adiposity in the rat by septal, preoptic and rostral hypothalamic lesions. A. W. Hetherington, Institute of Neurology, Northwestern University Medical School.
- Disturbances of sexual function in the female guinea pig following injury to the median eminence. F. L. Dey, Institute of Neurology, Northwestern University Medical School.
- Development of motor nuclei and fiber tracts after removal of large portions of the brain. Ruth Rhines, Department of Anatomy, Northwestern University Medical School. (Introduced by W. F. Windle.)
- Further studies on the origin of the ciliary ganglion. David S. Jones, Loyola University School of Medicine.
- The arterial pattern in the fore- and hind-limbs of the opossum. Arnold A. Zimmermann, Department of Anatomy, University of Illinois.

Demonstrations exhibited during the time of the meeting:

- a. The grooved nuclei of Brenner tumor cells. b. Origin of a Brenner tumor from the germinal epithelium of the ovary. L. B. Arey, Department of Anatomy, Northwestern University Medical School.
- Some of the more important features of the Huber six-somite human embryo. L. B. Arey and J. W. Henderson, Department of Anatomy, Northwestern University Medical School.
- The ossification of the modiolus. T. H. Bast, Department of Anatomy, University of Wisconsin.
- The precentral motor cortex of primates. Gerhardt von Bonin, Department of Anatomy, University of Illinois.
- Anatomy of the female pelvis and perineum. Arthur H. Curtis, Barry J. Anson, Tom Jones, Franklin L. Ashley and Lindsay E. Beaton, Department of Gynecology and Obstetrics and Department of Anatomy, Northwestern University Medical School; Illustration Studios, University of Illinois.
- Persistent pronephric tubule opening into cephalic end of coelom in a 9-10 mm. human embryo. Otto F. Kampmeier, Department of Anatomy, University of Illinois.
- The activity of small vessels in the wing membrane of living bats. Paul S. Nicoll, Department of Physiology, Indiana University Medical School. (Introduced by R. L. Jones.)

A simple quartz rod illuminator suitable for research or student use. William L. Simpson, The Barnard Free Skin and Cancer Hospital.

The mechanism of nasopharyngeal occlusion. Leon H. Strong, Department of Anatomy, University of Michigan.

The Philadelphia Sectional Meeting. At the invitation of The Wistar Institute of Anatomy and Biology, the Association members residing in Philadelphia and its immediate environs, Swarthmore and Bryn Mawr, organized an informal Sectional Meeting which was held at The Wistar Institute on Thursday, April 22 and Friday, April 23. With the exception of one paper and the address of the Acting President, the titles and abstracts of all communications were submitted to the Secretary of the Association in the regular way, and printed in the Abstract number of The Anatomical Record (vol. 85, no. 3, 1943). This procedure was adopted in order to ensure maintenance of the standards of the Association. There were 17 papers, 2 motion pictures, an address by the Acting President of the Association and informal demonstrations of research and other activities of The Wistar Institute by staff members. The informal Committee was made up of representatives of the various medical schools in Philadelphia, with J. P. Schaeffer, Chairman, E. J. Farris, Secretary and E. R. Clark, Program. Total registration was 34, of whom 27 were members of the Association, while a count of those attending the meetings reached the total of 261.

There were five visiting Anatomists from outside the Philadelphia Section, among them Prof. C. F. W. McClure and Prof. J. S. Nicholas. The latter, on request, spoke informally of the impact of the war on Anatomy. He urged Anatomists not to abandon their chosen lines of research, since results of value to war medicine and surgery were at least as likely to come from fundamental investigations as from research on specific war problems. He predicted that medical students in the near future would be decidedly less mature — 17 to 18 years old, with 18 months of college, requiring more skillful teaching than in the past. A further prediction was that there would be an increased need for medical men for 18 years after the end of the war.

At the end of the final session a resolution of appreciation of The Wistar Institute was adopted. Regarding future meetings the following motion was passed: "This group is of the opinion that local sectional meetings should be again authorized by the Executive Committee, in case national or regional meetings seem inadvisable." In the discussion, several, including Dr. Nicholas, favored a national meeting for next year. All agreed that the Philadelphia Sectional Meeting had been profitable, delightful and worth repeating. No especial luncheon, dinner or smoker arrangements were made.

The program was as follows:

Thursday, April 22, 2:00 p. m.

- The derivatives of the thalamus ventralis in the human brain. H. Kuhlenbeck, Woman's Medical College of Pennsylvania.
- Pigmentation of substantia nigra and locus caeruleus in certain carnivores. J. O. Brown, University of Pennsylvania. (Introduced by E. R. Clark.)
- The course and decussation of ectopic Mauthner's fibers in *Amblystoma punctatum*. J. Piatt, University of Pennsylvania.
- The hypophysis of the goose-fish (*Lophius pisc. L.*). W. H. F. Addison, University of Pennsylvania.
- The origin of the hypoglossal musculature in the cat. M. N. Bates, Daniel Baugh Institute of Anatomy, Jefferson Medical College.
- The drainage pathways of lymph from the thyroid gland (cat). A. J. Ramsay, Daniel Baugh Institute of Anatomy, Jefferson Medical College. (Illustrated by motion picture.)
- The formation of venae comites. E. R. Clark and Eleanor Linton Clark, University of Pennsylvania.
- Vascular reactions to histamine as seen with the microscope in living, unanaesthetized rabbits. R. G. Abell, University of Pennsylvania.

Demonstrations by The Wistar Institute Staff

Following the Thursday afternoon session for the reading of papers, members of the staff of The Wistar Institute demonstrated some of the phases of the research and other activities of the Institute. The demonstrations were presented by Dr. W. H. Lewis, Dr. Margaret Lewis, Dr. Helen D. King, Dr. Paul Aptekman, Dr. E. J. Farris, Dr. Eleanor Yeakel and Miss Meeser.

8:00 P.M.

Address by the Acting President of the Association, Dr. J. Parsons Schaeffer:
The incidence, types, and embryology of congenital atresias of the choanae.

This was followed by two motion pictures:

- The biology of reproduction in rats. E. J. Farris, The Wistar Institute of Anatomy and Biology. (Abstract in Anat. Rec., v. 84, no. 4, p. 481, 1942.)
- The formation of the blastodisc in the egg of the zebra fish, *Brachydanio rerio*. W. H. Lewis and E. C. Roosen-Runge, The Wistar Institute of Anatomy and Biology and Brown University.

Friday, April 23, 9:30 a. m.

- A morphological, experimental, and clinical study of the semitendineus muscle in various animals, including man. G. A. Bennett, Daniel Baugh Institute of Anatomy, Jefferson Medical College.
- The cricopharyngeus muscle. O. V. Batson, Graduate School of Medicine, University of Pennsylvania.
- Subdivision of the lung on the basis of bronchial distribution. J. F. Huber, Temple University Medical School.
- The variational anatomy of the hepatic and cystic arteries. N. A. Michels, Daniel Baugh Institute of Anatomy, Jefferson Medical College.
- The early development of the spino-sensory system in the albino rat. A. W. Angulo, Hahneman Medical School. (No abstract.)
- Effects of a neurophysiological stimulus on the breeding of albino rats. E. J. Farris and E. H. Yeakel, The Wistar Institute of Anatomy and Biology. (Abstract in Anat. Rec., v. 84, no. 4, p. 454, 1942.)
- Early implantation and shortened gestation in the marten. R. K. Enders and O. P. Pearson, Swarthmore College and U. S. Department of the Interior.
- The role of the superficial gel layer in gastrulation of the zebra fish egg. W. H. Lewis, The Wistar Institute of Anatomy and Biology.
- Trichomonas vaginalis* in tissue cultures. M. J. Hogue, University of Pennsylvania. (Illustrated by motion picture.)

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APPROVED MOTION PICTURE FILMS

The following films have been approved by one or more members of the Review Committees of the American Association of Anatomists or the American Society of Zoologists, and The Wistar Institute:

SUBJECT AND TITLE	LENGTH IN REELS (1-16 MM. REEL = 400 FT. RUNNING TIME ABOUT 15 MIN.)	PRODUCER	CONDITIONS FOR SECURING FILMS	DISTRIBUTOR
ANATOMY				
Congenital anomalies (monochrome, silent) (kodachrome, silent)	1	J. Sarnoff	Sale: Monochrome, \$25.00 Kodachrome, \$75.00	Sarnoff Motion Picture Film Library 1406 Albemarle Road Brooklyn, N. Y.
* The management of cica- tricial strictures of the esophagus. Treatment of cancer of the esopha- gus. Bronchoscopy with insufflation of sulfanil- amide in chronic bronchiectasis (kodachrome, silent)	1	E. H. Ingersoll	Rental, \$8.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* Surgical anatomy (kodachrome, silent)	4	C. J. Baumgartner	Sale Kodachrome	Billy Burke Productions 7416 Beverly Blvd. Hollywood, Calif.
BLOOD CELLS				
* 10. Normal and abnormal white blood cells in tissue cultures (monochrome, silent)	1	W. H. Lewis M. R. Lewis A. R. Rich M. M. Wintrobe	Rental, \$4.00 per day Sale, \$25.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
CANCER CELLS				
* 6. Dividing cancer cells in vitro (monochrome, silent)	160 ft.	W. H. Lewis M. R. Lewis	Rental, \$2.00 per day Sale, \$10.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* 3. Tumor cells and macro- phages in tissue cul- tures. Rat sarcomas and carcinomas (monochrome, silent)	360 ft.	W. H. Lewis	Rental, \$3.50 per day Sale, \$20.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
CIRCULATION				
* The control of small blood vessels (mono- chrome, silent)	1	G. P. Fulton B. R. Lutz	Rental or sale	Dr. G. P. Fulton College of Liberal Arts Boston University Boston, Mass.

SUBJECT AND TITLE	LENGTH IN REELS (1-16 MM. REEL = 400 FT. RUNNING TIME ABOUT 15 MIN.)	PRODUCER	CONDITIONS FOR SECURING FILMS	DISTRIBUTOR
<i>Circulation (Cont.)</i>				
* Effect of various drugs on contractions of mesenteric lymphatics in the rat (monochrome, silent)	300 ft.	R. L. Webb	Rental, \$3.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* The heart and circulation (monochrome, silent)	1	A. J. Carlson	Sale	Erpi Classroom Films, Inc. 35-11 35th Ave. Long Island City N. Y.
* Mesenteric lymphatics, their conduct and the behavior of their valves in the living rat (monochrome, silent)	1	R. L. Webb	Rental, \$4.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* Micromanipulative studies of blood capillaries (monochrome, silent)	1	B. W. Zweifach	Rental, \$4.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* 7. Growth of capillaries and endothelium from the sub-cutaneous tissue of 7-day chick embryos in 2-day cultures in Locke-bouillon-dextrose medium (monochrome, silent)	140 ft.	W. H. Lewis	Rental, \$2.00 per day Sale, \$9.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
CYTOLOGY				
* 8. Pinocytosis. Drinking by cells (monochrome, silent)	264 ft.	W. H. Lewis	Rental, \$3.00 per day Sale, \$16.50	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* 9. Dividing normal adult rat fibroblasts in vitro (monochrome, silent)	160 ft.	W. H. Lewis	Rental, \$2.00 per day Sale, \$10.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
EMBRYOLOGY				
* Activity of heart muscle, from rat embryos, grown in tissue culture (monochrome, silent)	1	C. M. Goss	Rental, \$4.00 Sale, \$25.00	Dr. C. M. Goss University of Alabama University, Ala.
Development of a salamander (monochrome, silent)	1	L. S. Stone and T. C. Kramer	Rental Sale	Films, Inc. 330 W. 42nd St. New York, N. Y.
* 1. The early development of the rabbit egg in vitro (monochrome, silent)	1	W. H. Lewis and P. W. Gregory	Rental, \$4.00 per day Sale, \$25.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* 4. The early development of the rabbit egg (short film — selected scenes from the long film) (monochrome, silent)	80 ft.	W. H. Lewis and P. W. Gregory	Rental, \$1.50 per day Sale, \$5.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.

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<i>Embryology (Cont.)</i>				
* 5. Early divisions, 2-8 cells, of living monkey egg in vitro (monochrome, silent)	80 ft.	W. H. Lewis and C. G. Hartman	Rental, \$1.50 per day Sale, \$5.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
Forming an egg (kodachrome, sound)	1	D. C. Warren and H. M. Scott	Rental, \$3.00 per day	Poultry Department Kansas State College Manhattan, Kans.
* Where chick life begins (kodachrome, silent)	1	A. L. Romanoff	Rental Sale	Ralston Purina Co. St. Louis, Mo.
* 11. Development of Zebra fish egg (monochrome silent)	1	W. H. Lewis and E. C. Roosen-Runge	Rental, \$4.00 per day Sale, \$25.00	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* Development of the opossum (kodachrome silent)	150 ft.	E. J. Farris	Rental, \$3.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
GENERAL				
Practical preventive orthodontics (kodachrome silent)	2	M. J. Futterman	Rental	Dr. M. J. Futterman 1749 Grand Concourse Bronx, N. Y.
* High speed motion pictures taken with stroboscopic light (monochrome, silent)	1	H. E. Edgerton K. J. Germeshausen H. E. Grier	Rental free Sale, \$25.00	Prof. Charles E. Locke Massachusetts Institute of Technology Cambridge, Mass.
* Seeing the unseen (monochrome, silent)	1	H. E. Edgerton	Rental free Sale, \$25.00	Prof. Charles E. Locke Massachusetts Institute of Technology Cambridge, Mass.
* Tropical opossums (kodachrome, silent)	1	E. J. Farris	Rental, \$8.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* Unseen worlds (monochrome, sound)	1	RCA Victor Camden, N. J.	Transportation charges only	Wm. J. Ganz Co. 19 E. 47 St. New York, N. Y.
NERVOUS SYSTEM				
* Effect of electrical stimulation of the right ansiform lobe of the cerebellum of the cat (<i>Felis domestica</i>) (monochrome, silent)	100 ft.	S. L. Clark	Rental	Dr. Sam L. Clark Department of Anatomy Vanderbilt University Nashville, Tenn.
* The eyes of science (monochrome, silent)	3	Bausch & Lomb Optical Co.	Rental free	Bausch & Lomb Optical Co. Rochester, N. Y.
* The nervous system (monochrome, silent)	14	I. V. Pavlov and others	Rental or sale	Brandon Films, Inc. 1600 Broadway New York, N. Y.

SUBJECT AND TITLE	LENGTH IN REELS (1-16 MM. REEL = 400 FT. RUNNING TIME ABOUT 15 MIN.)	PRODUCER	CONDITIONS FOR SECURING FILMS	DISTRIBUTOR
REPRODUCTION				
* The biology of reproduction in rats (kodachrome, silent)	1	E. J. Farris	Rental, \$8.00 per day	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
* Maternal behavior in the rat (monochrome, silent)	350 ft.	F. A. Beach	Rental, \$8.00 per day Sale, \$30.00	American Museum of Natural History New York, N. Y.
Mating behavior in the leopard frog, <i>Rana pipiens</i> (monochrome, silent)	250 ft.	F. A. Beach	Rental, \$8.00 per day Sale, \$20.00	American Museum of Natural History New York, N. Y.
Ovulation in the frog, <i>Rana pipiens</i> (monochrome, silent)	512 ft.	R. Rugh	Rental, \$5.00 per day Sale, \$25.00	Dr. Roberts Rugh N. Y. University Washington Square College New York, N. Y.
* Proestrous and oestrous behavior in the guinea pig (monochrome, silent)	150 ft.	W. C. Young E. W. Dempsey R. Hertz and H. L. Myers	Rental, \$1.50 per day Sale	The Wistar Institute 36th St. and Woodland Ave. Philadelphia, Pa.
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* Studies in human fertility	1 (2000 ft.)	Audio Productions, Inc.	Rental free	Ortho Products, Inc. Linden, N. J.
RESPIRATION				
Mechanisms of breathing (monochrome, sound)	1	V. Johnson	Sale	Erpi Classroom Films, Inc. 35-11 35th Ave. Long Island City N. Y.

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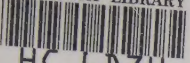
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